PATENT COOPERATION TREATY

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(PCT Article 20)

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CORRECTED VERSION
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PATENT COOPERATION TREATY

PCT **NOTIFICATION OF ELECTION** (PCT Rule 61.2)	From the INTERNATIONAL BUREAU To: United States Patent and Trademark Office (Box PCT) Washington D.C. 20231 United States of America in its capacity as elected Office
Date of mailing (day/month/year) 14 February 1996 (14.02.96)	
International application No. PCT/US95/07721	**Applicant's or agent's file reference** MIT 6620
International filing date (day/month/year) 19 June 1995 (19.06.95)	**Priority date** (day/month/year) 23 June 1994 (23.06.94)
Applicant KRIEGER, Monty et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   23 January 1996 (23.01.96)

   [ ] in a notice effecting later election filed with the International Bureau on:

   ____

2. The election [X] was
   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No.: (41-22) 740.14.35	Authorized officer Mirjam Van Straten Telephone No.: (41-22) 730.91.11

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PATENT COOPERATION TREATY

REC'D 27 SEP 1996
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference MIT 6620	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US 95/07721	International filing date (*day/month/year*) 19/06/1995	Priority date (*day/month/year*) 23/06/1994
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant MASSACHUSETTS INSTITUTE OF TECHNOLOGY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consists of a total of ______ sheets.

3. This report contains indications and corresponding pages relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand 23/01/1996	Date of completion of this report 26.09.96
Name and mailing address of the IPEA European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Netherlands Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer R. Hix Telephone No.

Form PCT/IPEA/409 (cover sheet) (January 1994) (12/03/1996)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT	International application No. PCT/US95/07721

I. Basis f the rep rt

1. This report has been drawn up on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*

   ☒ the international application as originally filed

   ☐ the description, pages , as originally filed
   pages , filed with the demand
   pages , filed with the letter of

   ☐ the claims, Nos. , as originally filed
   Nos. , as amended under Article 19
   Nos. , filed with the demand
   Nos. , filed with the letter of

   ☐ the drawings, sheets / fig. , as originally filed
   sheets / fig. , filed with the demand
   sheets / fig. , filed with the letter of

2. Th amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.

   ☐ the drawings, sheets / fig.

3. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2 (c)).

4. Additional observations, if necessary:

III. Non-establishment of pinion with r gard to novelty, inventiv st p and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 44 to 50

becaus :

☒ th said international application, or the said claims relate to the following subject matter which does not require an international preliminary examination *(specify)*: Nos. 44 to 50

Claims 44 to 50 involve methods of treatment of the human or animal body by therapy which encompass methods carried out in vivo, according to Article 34{4}{a}{i} and Rule 67.1{iv} PCT.

☐ th description, claims or drawings *(indicate particular elements below)* or said claims are so unclear that no meaningful opinion could be formed *(specify)*: Nos.

☐ th claims, or said claims are so inadequately supported by the description n meaningful opinion could be formed. Nos.

☐ no international search report has been established for said claims Nos.

IV. Lack of unity of inv nti n

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this r port:

☐ all parts.

☒ the parts relating to claims Nos.

Claims 1-8, 11-22 and partially Claims 9 and 10 : Scavenger receptor protein BI, nucleic acid sequence and antibodies. Methods of screening for scavenger receptor protein BI and removing low density lipoprotein from blood samples and inhibiting lipoprotein or lipid uptake by using or inhibiting the scavenger receptor protein BI.

V. Reas n d stat m nt under Article 35(2) with regard to n v lty, inventive st p r industrial applicability; citati ns and explanati ns supporting such statem nt

1. Statement

Novelty	Claims	3-5, 13-15	YES
	Claims	1, 2, 6-12, 16-22	NO
Inventive Step	Claims	1-22	YES
	Claims		NO
Industrial Applicability	Claims	1-22	YES
	Claims		NO

2. Citations and Explanations

The following documents are cited in this report;

DI : Nature, vol. 343, 8th February 1990, pages 531-535, T. Kodama et al.
DII : The Journal of Biological Chemistry, vol. 268, no. 7, 5th March 1993, pages 4569-4572, M. Krieger et al.
DIII : Journal of Clinical Investigation, vol. 93, no. 5, May 1994, pages 2014-2021, J. Luoma et al.
DIV : Biochemical Journal, vol. 304, no. 1, 15th November 1994, pages 69-73, Y. De Rijke et al.
DV : JP3290184 {Chugai Pharmaceutical Co. Ltd.}
DVI : JP5192179 {Chugai Pharmaceutical Co. Ltd.}
DVII : WO-A-9 005 748 {Massachusetts Institute of Technology}

1.} Independent product Claims 1 and 11 involve a scavenger receptor type BI characterised solely in terms of functional features {see Box VIII}. The term "type BI" is meaningless unless the proteins are characterised further in terms of technical features.

1.1} Consequently in the assessment of the novelty of the products of Claims 1 and 11 a comparison of the functional features of the product compared with those of the state of the art has been performed.

2.} DI discloses a bovine type I scavenger receptor protein, isolation from bovine alveolar macrophages and bovine lung, brain and muscle, determination of the amino acid and nucleic acid sequences and cloning of the cDNA. The scavenger receptor binds low density lipoprotein

{LDL} including modified LDL such as acetylated LDL.
2.1} The subject-matter of the vaguely defined Claims 1, 2, 11, 12, 16-18 and 20-22 is therefore not novel according to Article 33{2} PCT.

3.} DII involves the cloning and partial determination of the intron/exon organisation of genomic DNAs for human bovine and murine macrophage scavenger receptor which bind to modified acetylated LDL.

3.1} The subject-matter of Claims 1, 7, 8, 11 and 16-19 is not novel according to Article 33{2} PCT.

4.} DIII involves the characterisation of scavenger receptor proteins involved in the uptake of modified forms of LDL by macrophages and expressed in human lesion macrophages.

4.1} DIV also discloses human scavenger receptors from liver endothelial which are specific for modified LDL - oxidised and acetylated LDL.

4.2} In the light of the above two disclosures the subject-matter of Claims 1,2 and 7 is not novel according to Article 33{2} PCT.

5.} DV discloses the genes encoding type I and type II scavenger receptor in a vector used to transform animal host cells such as Chinese hamster ovary cells under the control of a promoter. The scavenger receptor proteins produced are used to detect modified lipoproteins or modified substances in blood.

5.1} Claims 1, 2, 7, 8, 11, 12, 16-19, 21 and 22 are not novel according to Article 33{2} PCT.

6.} DVI discloses an anti-human scavenger receptor antibody which is used as an antigen to immunise a mammal and isolate the antibody from the blood of the animal.
Claim 9 which is extremely broadly drafted is therefore not novel according to Article 33{2} PCT.

7.} DVII describes isolated scavenger receptors which bind to acetylated LDL and oxidized LDL, DNA sequences encoding at least a portion of the receptor protein and antibodies against the receptor protein, including methods of fixing the binding proteins to inert supports for purification and assay purposes, see page 23, second paragraph.

7.1} Claims 1, 2, 6, 7, 9, 10, 11 and 12 are not novel according to Article 33{2} PCT.

8.} Claims 3 to 5 and 13 to 15 involve the specific Sequence ID. No. of the scavenger receptor type protein with high affinity for modified lipoportiens and other ligands. The HaSR-BI, AcLDL and LDL binding scavenger receptor of the application, has been isolated, characterized and cloned from a variant of Chinese Hamster Ovary Cells. The scavenger receptor protein of the application, HaSR-BI is distinct from the type I and type II macrophage scavenger receptors known in the state of the art. Consequently the subject-matter of Claims 3 to 5 and 13 to 15, where the protein and nucleic acid are characterized by reference to the specific sequences involved, is considered to satisfy the requirements of novelty and inventive step according to Articles 33{2} and {3} PCT.

VIII. C rtain observations on the int rnational application

Th following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

1.} An independent claim must clearly define the essential technical features of the product, in this case, reference to the sequence ID of the amino acid or nucleic acid sequence encoding the protein is necessary in order to clearly and unambiguously characterise the claimed product. In their present form Claims 1, 9 and 11 do not satisfy the requirements of Article 6 PCT.

1.1} The above objection also applies to the independent method Claims 44, 48, 49 and 50, which refer to the scavenger receptor proteins in vague and general terms, characterising the products solely in terms of their functional features. Claims 44, 48, 49 and 50 are therefore also considered to contravene the requirements of Article 6 PCT.

2.} Claim 11 is rendered vague and imprecise through the combined use of the terms"at least fourteen nucleotides encoding at least in part...". Claim 11 in its present form contravenes the requirements of clarity according to Article 6 PCT.

3.} The term "consisting essentially of" used in Claims 5 and 15 renders the scope of the claim unclear and thus is not acceptable according to Article 6 PCT.

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US 08/265,428 (CIP)
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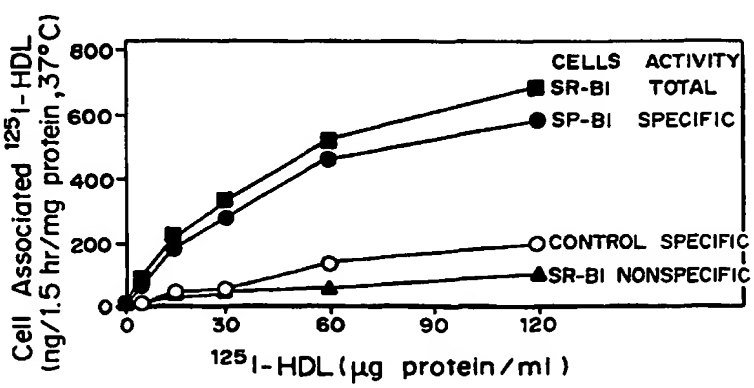
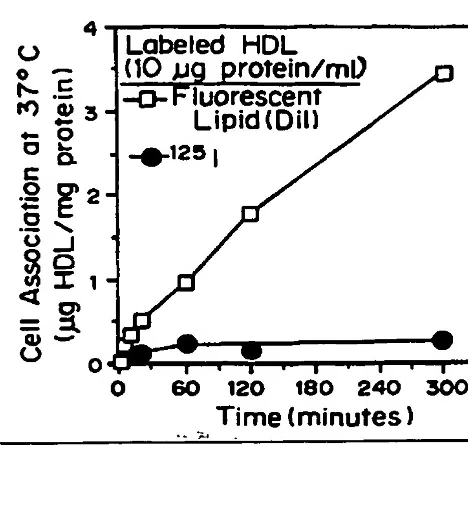
(54) Title: CLASS BI AND CI SCAVENGER RECEPTORS

(57) Abstract

Two distinct scavenger receptor type proteins having high affinity for modified lipoproteins and other ligands have been isolated, characterized and cloned. HaSR-BI, an AcLDL and LDL binding scavenger receptor, which is distinct from the type I and type II macrophage scavenger receptors, has been isolated and characterized and DNA encoding the receptor cloned from a variant of Chinese Hamster Ovary Cells, designated Var-261. dSR-CI, a non-mammalian AcLDL binding scavenger receptor having high ligand affinity and broad specificity, was isolated from *Drosophila melanogaster*. The isolated receptors are useful in screening for drugs that inhibit uptake of cholesterol in endothelial or adipose cells or macrophages, respectively. They are also useful as probes for the isolation of other lipoprotein receptors and in research the roles of these receptors.





CLASS BI AND CI SCAVENGER RECEPTORS

Background of the Invention

The present invention is generally in the area of new scavenger receptor proteins present on cells which can mediate lipid or lipoprotein uptake, genes encoding these proteins and methods of detection and use thereof.

The intercellular transport of lipids through the circulatory system requires the packaging of these hydrophobic molecules into water-soluble carriers, called lipoproteins, and the regulated targeting of these lipoproteins to appropriate tissues by receptor-mediated endocytic pathways. The most well characterized lipoprotein receptor is the LDL receptor, which binds to apolipoproteins B-100 (apoB-100) and E (apoE), which are constituents of low density lipoprotein, the principal cholesteryl-ester transporter in human plasma (LDL), very low-density lipoprotein, a triglyceride-rich carrier synthesized by the liver (VLDL), intermediate-density lipoprotein (IDL), and catabolized chylomicrons (dietary triglyceride-rich carriers synthesized by the liver).

All members of the LDL receptor gene family consist of the same basic structural motifs, shown in Figure 1a. Ligand-binding (complement-type) cysteine-rich repeats of approximately 40 amino acids are arranged in clusters (ligand-binding domains) that contain between two and eleven repeats. Ligand-binding domains are always followed by EGF-precursor homologous domains. In these domains, two EGF-like repeats are separated from a third EGF-repeat by a spacer region containing the YWTD motif. In LRP and gp330, EGF-precursor homologous domains are either followed by another ligand-binding domain or by a spacer region. The EGF-precursor homology domain, which precedes the plasma membrane, is separated from

the single membrane-spanning segment either by an O-linked sugar domain (in the LDL receptor and VLDL receptor) or by one (in *C. elegans* and gp330) or six EGF-repeats (in LRP). The cytoplasmic tails contain between one and three "NPXY" internalization signals required for clustering of the receptors in coated pits. In a later compartment of the secretory pathway, LRP is cleaved within the eighth EGF-precursor homology domain. The two subunits LRP-515 and LRP-85 (indicated by the brackets) remain tightly and non-covalently associated. Only partial amino acid sequence of the vitellogenin receptor and of gp330 are available.

LDL receptors and most other mammalian cell-surface receptors that mediate binding and, in some cases, the endocytosis, adhesion, or signaling exhibit two common ligand-binding characteristics: high affinity and narrow specificity. However, two additional lipoprotein receptors have been identified which are characterized by high affinity and broad specificity: the macrophage scavenger receptors type I and type II.

Scavenger receptors mediate the endocytosis of chemically modified lipoproteins, such as acetylated LDL (AcLDL) and oxidized LDL (OxLDL), and have been implicated in the pathogenesis of atherosclerosis (Krieger and Herz, 1994 J. Annu. Rev. Biochem. 63, 601-637; Brown and Goldstein, 1983 Annu. Rev. Biochem. 52, 223-261; Steinberg et al., 1989 N. Engl. J. Med. 320, 915-924). Macrophage scavenger receptors exhibit complex binding properties, including inhibition by a wide variety of polyanions, such as maleylated BSA (M-BSA) and certain polynucleotides and polysaccharides, as well as unusual ligand-cross competition (Freeman et al., 1991 Proc. Natl. Acad. Sci. U.S.A. 88, 4931-4935, Krieger and Herz, 1994). Several investigators have suggested that there may be at least three different classes of such receptors expressed on mammalian macrophages, shown in Figure 3, including

receptors which recognize either AcLDL or OxLDL, or both of these ligands (Sparrow et al., 1989 J. Biol. Chem. 264, 2599-2604; Arai et al., 1989 Biochem. Biophys. Res. Commun. 159, 1375-1382; Nagelkerke et al., 1983 J. Biol. Chem. 258, 12221-12227).

The first macrophage scavenger receptors to be purified and cloned were the mammalian type I and II receptors. These are trimeric integral membrane glycoproteins whose extracellular domains have been predicted to include α-helical coiled-coil, collagenous and globular structures (Kodama et al., 1990 Nature 343, 531-535; Rohrer et al., 1990; Krieger and Herz, 1994). The collagenous domain, shared by the type I and type II receptors, apparently mediates the binding of polyanionic ligands (Acton et al., 1993 J. Biol. Chem. 268, 3530-3537; Doi et al., 1993 J. Biol. Chem. 268, 2126-2133). The type I and type II molecules, which are the products of alternative splicing of a single gene, are hereafter designated class A scavenger receptors (SR-AI and SR-AII). The class A receptors, which bind both AcLDL and OxLDL (Freeman et al., 1991), have been proposed to be involved in host defense and cell adhesion, as well as atherogenesis (Freeman et al., 1991; Krieger, 1992 Trends Biochem. Sci. 17, 141-146; Fraser et al., 1993 Nature 364, 343-346; Krieger and Herz, 1994).

Models of the predicted quaternary structures of the type I and type II macrophage scavenger receptors are shown in Figure 1B (AR-A, I, II & III). Both contain six domains, of which the first five are identical: the N-terminal cytoplasmic region, the transmembrane region, spacer, α-helical coil, and collagen-like domains. The C-terminal sixth domain of the type I receptor is composed of an eight-residue spacer followed by a 102-amino acid cysteine-rich domain (SRCR), while the sixth domain of the type II receptor is only a short oligopeptide.

Using a murine macrophage cDNA library and a COS cell expression cloning technique, Endemann, Stanton

and colleagues, (Endemann, et al. 1993 J. Biol. Chem. 268, 11811-11816; Stanton, et al. J. Biol. Chem. 267, 22446-22451), reported the cloning of cDNAs encoding two additional proteins that can bind OxLDL. The binding of OxLDL to these proteins was not inhibited by AcLDL. These proteins are FcgRII-B2 (an Fc receptor) (Stanton et al., 1992) and CD36 (Endemann et al., 1993). The significance of the binding of OxLDL to FcgRII-B2 in transfected COS cells is unclear because FcgRII-B2 in macrophages apparently does not contribute significantly to OxLDL binding (Stanton et al., 1992). However, CD36 may play a quantitatively significant role in OxLDL binding by macrophages (Endemann et al., 1993). In addition to binding oxidized LDL, CD36 binds thrombospondin (Asch et al., 1987 J. Clin. Invest. 79, 1054-1061), collagen (Tandon et al., 1989 J. Biol. Chem. 264, 7576-7583), long-chain fatty acids (Abumrad et al., 1993 J. Biol. Chem. 268, 17665-17668) and *Plasmodium falciparum* infected erythrocytes (Oquendo et al., 1989 Cell 58, 95-101). CD36 is expressed in a variety of tissues, including adipose, and in macrophages, epithelial cells, monocytes, endothelial cells, platelets, and a wide variety of cultured lines (Abumrad et al., 1993; and see Greenwalt et al., 1992 Blood 80, 1105-1115 for review). Although the physiologic functions of CD36 are not known, it may serve as an adhesion molecule due to its collagen-binding properties. It is also been proposed to be a long-chain fatty acid transporter (Abumrad et al., 1993) and a signal transduction molecule (Ockenhouse et al., 1989 J. Clin. Invest. 84, 468-475; Huang et al., 1991), and may serve as a receptor on macrophages for senescent neutrophils (Savill et al., 1991 Chest 99, 7 (suppl)).

Modified lipoprotein scavenger receptor activity has also been observed in endothelial cells (Arai et al., 1989; Nagelkerke et al., 1983; Brown and Goldstein, 1983; Goldstein et al., 1979 Proc. Natl. Acad. Sci. U.S.A. 76, 333-337). The endothelial cell

activity apparently is not mediated by the class A scavenger receptors (Bickel et al., 1992 J. Clin. Invest. 90, 1450-1457; Arai et al., 1989; Nagelkerke et al., 1983; Via et al., 1992 The Faseb J. 6, A371), which are expressed almost exclusively by macrophages (Naito et al., 1991 Am. J. Pathol. 139, 1411-1423; Krieger and Herz, 1994). *In vivo* and *in vitro* studies suggest that there may be scavenger receptor genes expressed in endothelial cells and macrophages which differ from both the class A scavenger receptors and CD36 (Haberland et al., 1986 J. Clin. Inves. 77, 681-689; Via et al., 1992; Sparrow et al., 1989; Horiuchi et al., 1985 J. Biol. Chem. 259, 53-56; Arai et al., 1989; and see below). Via, Dressel and colleagues (Ottnad et al., 1992 Biochem J. 281, 745-751) and Schnitzer et al. 1992 J. Biol. Chem. 267, 24544-24553) have detected scavenger receptor-like binding by relatively small membrane associated proteins of 15-86 kD. In addition, the LDL receptor related protein (LRP) has been shown to bind lipoprotein remnant particles and a wide variety of other macromolecules. Both the mRNA encoding LRP and the LRP protein are found in many tissues and cell types (Herz, et al., 1988 EMBO J. 7:4119-4127; Moestrup, et al., 1992 Cell Tissue Res. 269:375-382), primarily the liver, the brain and the placenta. The predicted protein sequence of the LRP, shown in Figure 1A, consists of a series of distinctive domains or structural motifs, which are also found in the LDL receptor.

Based on the information known regarding the structures and functions of multiligand lipoprotein receptors present on macrophages, it would clearly be of benefit to isolate and clone other members of the lipoprotein receptor family present on macrophages, especially from non-mammalian species, in order to investigate which aspects of these molecules are most conserved and which portions can therefore be selectively targeted for stimulation or inhibition of

binding, and on other cell types, the structure and function of whose receptors are not characterized.

It is therefore an object of the present invention to provide the structure, amino acid sequence, and DNA sequence encoding a previously undescribed lipoprotein receptors present on mammalian cells.

It is another object of the present invention to provide the structure, amino acid sequence, and DNA sequence encoding a lipoprotein receptor present on insect macrophages.

It is a further object of the present invention to provide methods and reagents for use in isolating and characterizing lipoprotein receptors that are not type I and type II macrophage scavenger receptors nor classic LDL receptors.

It is yet a still further object of the present invention to provide methods and reagents for designing drugs that can stimulate or inhibit the binding of lipoprotein receptors that are not type I and type II macrophage scavenger receptors nor classic LDL receptors.

It is still another object of the present invention to provide a method and means for altering cholesterol uptake and transport by cells.

Summary of the Invention

Two distinct scavenger receptor type proteins having high affinity for modified lipoproteins and other ligands have been isolated, characterized and cloned. HaSR-BI, an AcLDL and LDL binding scavenger receptor, which is distinct from the type I and type II macrophage scavenger receptors, has been isolated and characterized and DNA encoding the receptor cloned from a variant of Chinese Hamster Ovary Cells, designated Var-261, and from murine cells. dSR-CI, a non-mammalian AcLDL binding scavenger receptor having

high ligand affinity and broad specificity, was isolated from *Drosophila melanogaster*.

It has been discovered that the SR-BI receptor is expressed principally in steroidogenic tissues and adrenal tissue and appears to mediate HDL-transfer and uptake of cholesterol. Competitive binding studies show that SR-BI binds LDL, modified LDL, negatively charged phospholipid, and HDL. Direct binding studies show that SR-BI binds HDL-lipid, without degradation of the HDL, and lipid is accumulated within cells expressing the receptor. These studies indicate that AR-BI plays a major role in transfer of cholesterol from the liver to the steroidogenic tissues, and that increased expression in the liver or other tissues may be useful in increasing uptake of cholesterol by cells expressing SR-BI, thereby decreasing levels in foam cells and deposition at sites involved in atherogenesis.

The presence of scavenger receptors on both mammalian and *Drosophila* macrophages indicates that they mediate critical, well-conserved functions, including pathogen recognition, and that they may have appeared early in the evolution of host defense systems. In this regard, it is known that postembryonic macrophage-like hemocytes in *Drosophila* participate in wound healing, encapsulation of pathogens, and phagocytosis. Due to the known association between atherosclerosis and macrophages, and the uptake of cholesterol by macrophages which is mediated by scavenger receptor proteins, the isolated receptors are useful in screening for drugs that inhibit uptake of cholesterol by cells expressing these receptors. Studies also demonstrate that a 600 bp *Bam*HI portion of the cDNA encoding SR-BI hybridizes under stringent conditions to a mRNA expressed in adipocytes. The hybridizing sequence does not express a protein immunoreactive with antibody to SR-BI. The protein encoded by the hybridizing sequence is referred to as SR-BII.

Brief Description of the Drawings

Figure 1a is a schematic of the LDL receptors described in the background of the invention. Figure 1b is a schematic of the three classes of scavenger receptors, SR-A (I, II and III), SR-B (CD36), and SR-C.

Figures 2A, 2B, and 2C are graphs of the concentration dependence of ^{125}I-AcLDL interaction with control cells, Var-261 and ldlA-7 (LDL receptor deficient control cells, also referred to as ldlA cells) cells, at 4°C and 37°C. Figure 2A is binding at 4°C; Figure 2B is binding plus uptake at 37°C; and Figure 2C is degradation at 37°C, measured as ng ^{125}I-AcLDL/5 hr/mg cell protein versus ^{125}I-AcLDL (μg protein/ml).

Figures 3A and 3B are graphs of the binding of ^{125}I-AcLDL to haSR-BI expressed in transfected COS cells which do not otherwise express SR-BI (Figure 3A) and specificity of binding of ^{125}I-AcLDL to Var-261 and ldlA [haSR-BI] cells (Figure 3B) measured as the percent of control binding in the presence of competitor: M-BSA (10 μg/ml), poly G (500 μg/ml), Fucoidin (200 μg/ml), Carrageenan (200 μg/ml), and LDL (500 μg/ml).

Figures 4A and 4B are graphs of the binding of ^{125}I-AcLDL to CD36 expressed in transfected COS cells (Figure 4A) and specificity of binding of ^{125}I-AcLDL to transfected COS cells (Figure 4B) measured as percent of control in the presence of a competitor: M-BSA (4 μg/ml), AcLDL (500 μg/ml), Fucoidin (200 μg/ml), Poly I (500 μg/ml), Poly G (500 μg/ml), and ReLPS (250 μg/ml).

Figure 5 is a graph of lipoprotein inhibition of ^{125}I-AcLDL binding to haSR-BI and huCD36, measured as percent of control binding at 4°C, to either COS[haSR-BI] or COS[huCD36], alone, in the presence of AcLDL, OxLDL or LDL.

Figures 6A, 6B, and 6C are graphs of the concentration dependent binding, uptake and degradation of ^{125}I-AcLDL to CHO[dSR-CI]-2.6a cells: Figure 6A is binding at 4°C; Figure 6B is binding plus uptake at 37°C; and Figure 6C is degradation at 37°C, measured as ng ^{125}I-AcLDL/5 hr/mg cell protein versus ^{125}I-AcLDL (μg protein/ml).

Figures 7a and 7b are graphs ^{125}I-AcLDL binding, 4°C (% of control) as a function of liposome concentration (μg phospholipid/ml)(PS, circles; PI, triangles; PC, open circles), haSR-BI, Figure 7a; huCD36, Figure 7b.

Figure 7c is a graph of the effect of PS:PC ratio on inhibition of ^{125}I-LDL binding to SR-BI, ^{125}I-LDL binding at 4°C (% of control) to PS in liposomes (mole %).

Figure 7d is [^{3}H]-liposome binding to haSR-BI, [^{3}H]-liposome binding at 4°C (μg phospholipid/mg cell protein) versus [^{3}H]-liposomes (μg phospholipid/ml) ([^{3}H]PS, ldlA[haSR-BI] (circles); [^{3}H]PS, ldlA (square); [^{3}H]PC, ldlA[haSR-BI] (open circles)).

Figure 7e is a graph of the specificity of binding to SR-BI, binding of haSR-BI at 4°C (% of control) versus competitor (150 μg/ml), [^{3}H]PS (light bar) and ^{125}I-LDL (dark bar).

Figure 8a is a graph of the cell associated ^{125}I-HDL (ng/1.5 hr/mg protein, 37°C) as a function of ^{125}I-HDL (μg protein/ml), for SR-BI transfected CHO cells and untransfected controls (ldlA-7).

Figure 8b is a graph of SR-BI mediated delivery of fluorescent lipid from HDL to cells expressing SR-BI on their surface, cell association at 37°C (μg HDL/mg protein) as a function of time (minutes), for fluorescent lipid-labeled HDL (squares) and ^{125}I-labeled HDL (circles).

Figure 9 is a schematic of potential roles of SR-BI in HDL metabolism.

Detailed Description of the Invention

In order to isolate, characterize, and clone the genes for new scavenger receptors and other lipoprotein receptors, cDNA and genomic libraries are prepared from cells in which activities have been identified which are characteristic of scavenger receptors: binding to lipoproteins such as LDL, HDL, AcLDL and/or oxidized-LDL; hybridization screening of the genomic libraries using probes generated from the nucleic acid sequences of cloned receptors; expression cloning by transient expression in COS cells and/or expression cloning by stable expression in CHO cells; analysis of the cloned cDNA: verification, sequencing and sequence analysis; identification and isolation of the genomic DNA including regulatory sequences; immunochemical analysis of the structure and biosynthesis of the new scavenger receptors; and characterization of the binding properties of the new receptors and comparisons with type I and type II receptors.

I. Isolation and Characterization of a mammalian scavenger receptor protein on CHO cells.

To extend the analysis of the structure and function of mammalian modified lipoprotein scavenger receptors, a variant Chinese hamster ovary cell line, Var-261, which, based on ligand specificity, expresses an apparently novel polyanion binding scavenger receptor, was identified and characterized. The cDNA for a scavenger receptor, haSR-BI, which is a new member of the CD36 family of membrane proteins (class B scavenger receptors), was isolated from the Var-261 cells. Although isolated from the same cells, haSR-BI is not responsible for the novel polyanion binding receptor activity of Var-261 cells, and is present in normal cells present in a variety of tissues, as discussed below.

The ligand specificities of CD36 and haSR-BI expressed in transfected cell lines was compared with that of Var-261 cells. haSR-BI differs from CD36 and

other modified lipoprotein receptors described to date in that its binding of AcLDL is inhibited by native LDL. SR-BI also binds HDL and mediates uptake of lipid from HDL into the cell.

The cDNA encoding SR-BI yields a predicted protein sequence of 509 amino acids which is approximately 30% identical to those of the three previously identified CD36 family members. Northern blot analysis of murine tissues shows that SR-BI is most abundantly expressed in fat and is present at moderate levels in lung and liver. Furthermore, SR-BI mRNA expression is induced upon differentiation of 3T3-L1 cells into adipocytes. Both SR-BI and CD36 display high affinity binding for acetylated LDL with an apparent dissociation constant in the range of approximately 5 μg protein/ml. The ligand binding specificities of CD36 and SR-BI, determined by competition assays, are similar, but not identical: both bind modified proteins (acetylated LDL, maleylated BSA), but not the broad array of other polyanions (e.g. fucoidin, polyinosinic acid, polyguanosinic acid) which are ligands of the class A receptors. SR-BI displays high affinity and saturable binding of HDL which is not accompanied by cellular degradation of the HDL. HDL inhibits binding of AcLDL to CD36, suggesting that it binds HDL, similarly to SR-BI. Native LDL, which does not compete for the binding of acetylated LDL to either class A receptors, CD36 or Var-261 cells, unexpectedly competes for binding to SR-BI. SR-BI and CD36 therefore define a second class of scavenger receptors, designated class B, which are referred to as members of the CD36 family which can bind to modified LDL. The ability of other known members of the CD36 family to bind to modified LDLs has not been reported. Class B scavenger receptors may play a role in the *in vivo* and *in vitro* uptake of modified proteins previously described by Haberland et al., 1989 J. Immunol. 142, 855-862; Villaschi et al., 1986 Microvasc. Res. 32, 190-199;

Horiuchi et al., 1985; Predescu et al., 1988 J. Cell Biol. 107, 1729-1738).

These methods and conclusions are described in greater detail below. Abbreviations: LDL (low density lipoprotein), OxLDL (oxidized LDL), AcLDL (acetylated LDL), M-BSA (maleylated BSA), CHO (Chinese hamster ovary), haSR-BI (hamster scavenger receptor type BI), mSR-AII (murine scavenger receptor type AII), huCD36 (human CD36).

Materials and Methods

Materials

LDL, AcLDL, ^{125}I-labeled AcLDL (100-400 cpm/ng protein), and newborn calf and human lipoprotein-deficient sera were prepared as described by Goldstein et al., 1983 Methods Enzymol. 98, 241-260; Krieger, 1983 Cell 33, 413-422. For some preparations of LDL and AcLDL, the following additional precautions were taken to prevent inadvertent oxidation: 1) 20 μM β-hydroxytoluene was added to the plasma before separation of the lipoproteins on the gradient, 2) one hour prior to all dialysis steps the dialysis solutions were degassed by vacuum, followed by purging with nitrogen gas 3) whenever possible purification procedures were performed in the dark or low light, and 4) the preparations were stored in the dark under argon. OxLDL was prepared by dialyzing 1 ml of LDL (4 -10 mg/ml) (prepared without β-hydroxytoluene) against saline solution containing 5 μM Cu_2SO_4 (2 x 500 ml) for 24 to 48 hours at 4°C. Using a lipid peroxidation assay (El-Saadani et al., 1989), very little inadvertent oxidation in LDL and AcLDL preparations isolated with or without the precautions listed above was measured. Polyriboguanylic acid (poly G), polyriboinosinic acid (poly I), fucoidin, dextran sulfate, chondroitin sulfate, ReLPS (*S. minnesota* Re-595) and carrageenan (type III Kappa) were obtained from Sigma Chemical Co. Maleylated BSA (M-BSA) was prepared as described by Goldstein et al., 1979. The CD36 expression vector was a gift from B. Seed

(Massachusetts General Hospital, Boston). 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) and 3-pyrenemethyl-23,24-dinor-5-cholen-22-oate-3b-yl oleate (PMCAO) were obtained from Molecular Probes (Eugene, OR) and were used to prepare fluorescently labeled AcLDL as described by Krieger et al., 1979 J. Supra. Struct. 10, 467-478; Krieger, 1986 Meth. Enzymol. 128, 608-613; Pitas et al., 1985 J. Cell. Biol. 100, 103-117; Kingsley and Krieger, 1984 Proc Natl. Acad. Sci. USA 88, 7844-7848. Compactin and dioctadecylamidoglycylspermine (DOGS) were generous gifts from A. Endo and J. R. Falck, respectively.

Cell culture and transfections.

CHO, ldlA (clone 7) and COS M6 cells were grown in culture as described by Krieger et al., 1983 Proc. Natl. Acad. Sci. USA 80, 5607-5611; Acton et al., 1993. All incubations with cells were performed at 37°C in a humidified 5% CO_2/95% air incubator unless otherwise noted. COS M6 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine (medium A). For transient transfections other than in the expression cloning experiments, 1 x 10^6 COS cells were plated in 100 mm dishes in medium A on day 0. On day 1, plasmid DNA (between 2 and 10 μg in 1.9 ml phosphate buffered saline [PBS, calcium/magnesium free]) was mixed with 100 μl of 10 mg/ml DEAE-dextran (Pharmacia) and then added to each dish, which was then incubated at 37°C with gentle shaking every 10 minutes. After 30 minutes, 8 ml of medium A supplemented with 80 μM chloroquine (Sigma) were added and the cells were incubated for another 2.5 hrs. The medium was removed and the cells were shocked with 5 ml of 10% (v/v) dimethylsulfoxide in medium A for 2.5 minutes. This medium was quickly removed by aspiration, the cells were washed with 10 ml of PBS (containing calcium and magnesium), and 10 ml of fresh

medium A were added. On day 2 the cells were harvested with trypsin and replated (0.5 - 1 x 10^6 cells/well in 6-well dishes) in medium A containing 1 mM sodium butyrate. On day 3, the cells were assayed for ligand binding at 4°C and for binding plus uptake (binding/uptake) and degradation at 37°C. Stable transfections were performed as follows: ldlA cells were plated at 1 x 10^6 cells per 100 mm dish in medium B (Ham's F12 containing 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine) supplemented with 10% fetal calf serum. On day 2, the cells were washed three times with medium B. A complex of DNA and DOGS (dioctadecylamidoglycylspermine, which was a gift from J.R. Falck) was prepared by adding 150 μl of a DNA solution (12 μg of plasmid and 1.2 μg of supercoiled pSV2neo in 300 mM NaCl) to 150 μl of solution A (0.33 mg/ml DOGS, 300 mM NaCl). The DNA/DOGS complex was added to the cells in 3.6 ml of serum-free Opti-MEM medium (GIBCO) containing penicillin (100 U/ml) and streptomycin (100 μg/ml). After a 14 hr transfection period, the cells were washed three times with medium B and incubated in medium B plus 10% fetal calf serum for one day before being replated at a density of 3 x 10^6 cells per 100 mm dish. One day later, the culture medium was replaced with selection medium, medium B supplemented with 10% fetal calf serum and 0.25 mg/ml G418. This medium was changed every two to three days, and G418-resistant colonies appeared 10 to 12 days after transfection. The colonies were screened for DiI-AcLDL endocytosis and positive colonies were picked and grown to mass culture for further analysis.

Isolation of Var-261 cells.

Var-261 cells, a rare spontaneously arising variant expressing a novel scavenger receptor activity, were isolated during a transfection/selection experiment as follows. Human liver genomic DNA prepared as described by Sege et al., 1984 Nature 307, 742-745, was transfected into

ldlA (clone 7) cells, a CHO cell mutant clone whose defective LDL receptor gene results in an essentially LDL receptor-negative phenotype (Sege, et al., 1986 Mol. Cell. Biol. 6, 3268-3277; Kingsley et al., 1986 Mol. Cell. Biol. 6, 2734-2737; Kingsley and Krieger, 1984). After transfection, variants expressing scavenger receptor activity were isolated by growing the cells in MAC selection medium (Penman et al., 1991 J. Biol. Chem. 266, 23985-23993) [250 μM Mevalonate, 3 μg protein/ml of AcLDL, 40 μM Compactin, and 3% (v/v) newborn calf lipoprotein-deficient serum in medium B. In MAC medium, endogenous cholesterol synthesis is inhibited by compactin and only those cells that are able to obtain cholesterol by the endocytosis of AcLDL can survive. The parental ldlA cells used for the transfection cannot grow in MAC medium. After incubation in MAC medium for 29 days, surviving colonies were re-fed with medium B supplemented with 3% (v/v) newborn calf lipoprotein-deficient serum containing AcLDL which was fluorescently labeled by reconstitution of the lipid core (Krieger, 1986) with the pyrene-based lipophilic dye PMCAO (Krieger et al., 1979). Colonies which accumulated significant amounts of fluorescence from the lipoprotein, as determined by fluorescence microscopy (Krieger et al., 1981 J. Mol. Biol. 150, 167-184), were harvested and maintained in MAC selection medium. One colony, designated Var-261, exhibited significant levels of AcLDL binding, uptake and degradation activity which were dramatically greater than those in either the parental ldlA cells or any of the other colonies. Southern blot analysis using human genomic DNA as a probe showed that there was no detectable human-specific repeat DNA in Var-261 cells. Thus, Var-261 cells are presumably rare, spontaneously arising variants which express an endogenous hamster scavenger receptor gene. This expression could be due to the activation of an otherwise silent gene. Alternatively, it might be due to a spontaneous mutation which conferred novel

activity on an endogenous protein with some other function (e.g., see Faust and Krieger, 1987 J. Biol. Chem. 262, 1996-2004, and Chen et al., 1990 J. Biol. Chem. 265, 3116-3123).

^{125}I-AcLDL Binding, Uptake and Degradation Assays. Scavenger receptor activities at 37°C were measured by ligand binding, uptake and degradation assays as described by Krieger, 1983; Freeman et al., 1991). Although binding and uptake at 37°C were determined separately using dextran-sulfate to free surface bound lipoproteins from the cells as described for LDL receptor assays by Goldstein et al., 1983; Basu et al., 1983 Science 219, 871-873), this method for separating bound from internalized ligand for ^{125}I-AcLDL and scavenger receptors has not been independently validated. Accordingly, the values for binding and uptake were combined and are presented as binding plus uptake observed after a 5 hour incubation and are expressed as ng of ^{125}I-AcLDL protein per 5 hr per mg cell protein. Degradation activity is expressed as ng of ^{125}I-AcLDL protein degraded in 5 hours per mg of cell protein. The specific, high affinity values presented represent the differences between the results obtained in the presence (single determinations) and absence (duplicate determinations) of excess unlabeled competing ligand (75 to 200 μg/ml of M-BSA). Cell surface 4°C binding was assayed using either method A or method B as indicated. In method A, cells were prechilled on ice for 15 min, re-fed with ^{125}I-AcLDL in ice-cold medium B supplemented with 10% (v/v) fetal bovine serum, with or without 75 - 200 μg/ml unlabeled M-BSA, and incubated 2 hr at 4°C on a shaker. Cells were then washed rapidly three times with Tris wash buffer (50 mM Tris-HCl, 0.15 M NaCl, pH 7.4) containing 2 mg/ml BSA, followed by two 5 min washes, and two rapid washes with Tris wash buffer without BSA. The cells were solubilized in 1 ml of 0.1 N NaOH for 20 min at room temperature on a shaker, 30 μl were removed for protein determination, and the

radioactivity in the remainder was determined using a LKB gamma counter. Method B differed from method A in that the cells were prechilled for 45 minutes, the medium contained 10 mM HEPES and 5% (v/v) human lipoprotein-deficient serum rather than fetal bovine serum, and the cell-associated radioactivity released by treatment with dextran sulfate was measured as described by Krieger, 1983; Freeman et al., 1991).

Preparation of Var-261 cDNA library.

Poly A^+ mRNA was isolated from Var-261 cells using standard procedures (Sambrook, Fritsch, and Maniatis. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press (1989)). This mRNA was used to synthesize double-stranded cDNA using a Not I unidirectional primer (Invitrogen) according to the method of Aruffo and Seed, 1987 Immunology 84, 8573-8577. BstXI linkers (Invitrogen) were added to the cDNA. The isolated cDNA was digested with Not I, size selected from agarose gels (in three size groups: 1 to 2 kb, 2 to 4 kb, greater than 4 kb), and ligated into the expression vector pcDNA I (Invitrogen). The average insert size for the entire Var-261 unidirectional library was approximately 1.5 kb. The DNAs for transfection experiments were prepared as follows. *E. coli* strain MC1061/P3 was transformed with the cDNA expression plasmids by electroporation and the transformed cells were plated on LB-A/T plates (Luria broth with ampicillin (15 μg/ml)/tetracycline (8 μg/ml)) (Sigma) to obtain isolated colonies at densities of approximately 5,000 per 150 mm dish. Each plate, representing one pool, was scraped to recover the bacteria and a pool of library plasmids was isolated from each bacterial mixture using a midiprep method (miniprep method scaled up 5 to 10 fold (Sambrook et al., 1989)).

Expression cloning by transient expression in COS cells.

On day 0, COS M6 cells were plated in 35 mm culture dishes (3 to 4 x 10^5 cells/dish) in medium A.

On day 1, the cells in each dish were transfected with 0.5 μg/dish of expression library DNA from a single pool following the DEAE-dextran method of Cullen 1987 Methods in Enz. 152, 684-704. On day 2, monolayers were re-fed with medium A containing 1 mM sodium butyrate (modified medium A). On day 3, the monolayers were re-fed with modified medium A containing one to five μg protein/ml of DiI-labeled AcLDL (DiI-AcLDL). After a 5 hr incubation at 37°C, the plates were washed two times with PBS and the cells were fixed with 3.7% formaldehyde in PBS for 10 min at room temperature. The presence of fluorescent DiI in the fixed cells was determined by visual inspection using a Leitz inverted fluorescence microscope with a rhodamine filter package as described by Kingsley and Krieger, (1984). Each transfection experiment included negative control transfections with empty vector pcDNA I (0 to 20 DiI-positive cells per dish) and positive control transfections with a mixture of a pcDNA I-based bovine type II macrophage scavenger receptor expression vector, pXSR3 (Rohrer et al., 1990) and the empty vector pcDNA I (1:5000 ratio, 200 to 400 DiI-positive cells per dish). A positive pool was serially subdivided and retested to permit the purification of the single positive plasmid, phaSR-BI.

Northern blot analysis.

0.5 micrograms of poly(A)+ RNA prepared from different murine tissues or from 3T3-L1 cells on zero, two, four, six or eight days after initiation of differentiation into adipocytes as described by Baldini et al., 1992 Proc. Natl. Acad. Sci. U.S.A. 89, 5049-5052, was fractionated on a formaldehyde/agarose gel (1.0%) and then blotted and fixed onto a Biotrans™ nylon membrane. The blots were hybridized with the indicated probes that were ^{32}P-labeled (2 x 10^6 dpm/ml, random-primed labeling system). The hybridization and washing conditions, at 42°C and 50°C, respectively, were performed as described by

Charron et al., 1989 Proc. Natl. Acad. Sci. U.S.A. 86, 2535-2539. The probe for haSR-BI mRNA analysis was a 0.6 kb BamHI fragment from the cDNA's coding region. The coding region of murine cytosolic hsp70 gene (Hunt and Calderwood, 1990 Gene 87, 199-204) was used as a control probe for equal mRNA loading.

SR-BI protein in tissues was detected by blotting with polyclonal antibodies to SR-BI.

HDL Binding Studies

HDL and VLDL binding to SR-BI and CD36 were conducted as described for LDL and modified LDL.

Studies conducted to determine if the HDL which is bound to SR-BI is degraded or recycled and if lipid which is bound to the HDL is transferred into the cells were conducted using fluorescent lipid-labeled HDL and ^{125}I-HDL added to cultures of transfected or untransfected ldlA-7 at a single concentration (10 μg protein/ml). HDL associated with the cells was measured over time. A steady state was reached in approximately thirty minutes to one hour. A fluorescent ligand, DiI, described above with reference to LDL, was used as a marker for lipid (for example, cholesterol or cholesterol ester) uptake by the cell. Increasing concentration of DiI indicates that lipid is being transferred from the HDL to the receptor, then being internalized by the cell. The DiI-depleted HDL is then released and replaced by another HDL molecule bound to lipid.

Phospholipid Binding and Competition Assays

Materials- Reagents (and sources) were acetic subhydride (Mallinckrodt, Inc., Paris, KY); egg phosphatidylcholine, egg phosphatidic acid, liver phosphatidylinositol, brain phosphatidylserine, egg phosphatidylethanolamine, and brain sphingomyelin (Avanti Polar Lipds, Inc., Alabaster, AL); polycarbonate membrane filters (Poretica Corp., Livermore, CA); sodium [^{125}]iodide and 1,2-dipalmitoyl-L-3-phosphatidyl [*N-methyl*-^{3}*H*] choline ([^{3}H]DPPC) (Amersham Corp.); DEAE-dextran (Pharmacia

Biotech. Inc., Ham's F-12 medium, Dulbecco's modified Eagle's medium, fetal bovine serum, and trypsin/EDTA ((JRII Bio-science, Lenera, KS); and penicillin/streptomycin, glutamine, and GIBCO BRL G-418 sulfate (Life Technologies, Inc.). All other reagents and supplies were purchased from Sigma or were obtained as described previously (Krieger, 1983). Human LDL, AcLDL, ^{125}I-labeled LDL, and ^{125}I-labeled AcLDL (90-300 cpm/ng protein) were prepared essentially as described previously (Goldstein *et al.*, 1983; Krieger, 1983; Acton *et al., 1994)*.

Phospholipid Liposome Preparation. Unilamellar liposomes were made by extrusion through policarbonate membranes (Szoka *et al.*, 1980). Phospholipid liposomes were prepared containing the indicated phospholipid, phosphatidycholine, and free cholesterol in a molar ratio of 1:1:1. The lipids were mixed in chloroform and dried by rotary evaporation for 30 min. For preparation of radiolabeled liposomes, 50-75 μCi of [^{3}H]DPPC(62 Ci/mmol) were added to the lipid mixtures before drying. The dried lipids were resuspended in 150 mM NaCl, 0.1 mM EDTA, 10 mM HEPES, pH 7.5 (Buffer A). Once the samples were fully hydrated, they were extruded through 0.1-μ pore size polycarbonate membranes using a mini-extruder device (Avanti Polar Lipids, Inc., Alabaster, AL). After extrusion, liposomes were dialyzed against Buffer A and then stored under nitrogen at 4°C until use. Liposomes were used within 2 weeks of preparation. The final phospholipid concentration was determined by the method of Bartlett (1959). The average diameters of unlabeled liposomes, which were determined from either two or three independent preparations using light scattering with a Coulter N4 plus light scatterer apparatus (Coulter Electronics Inc., Hialeah, FL), were: PS, 105; PC, 114: PA, 125; PE, 129; P1, 113; and SM, 131 nm. The number of phospholipid molecules/PS liposome was calculated as follows. Cross-sectional

areas for cholesterol and phospholipid molecules in hydrated bilayers are assumed to be 0.35 nm^2 and 0.47 nm^3, respectively (Levine and Wilklus, 1971); assuming an homogenous distribution of the components throughout the PS/PC/cholesterol (1:1:1) liposomes, 73% of the surface area ($4\Pi r^2 x2$ (bilayer) x 0.73 = 50477 nm^2) was phospholipid, or 107,398 phospholipid molecules/liposome (50477 nm^2/0.47nm^2). Based on an average phospholipid mass of 785 g/mol, a liposome concentration of 10 μg phospholipid/ml converts to 0.12 nM in liposome particles.

Ligand Binding Assays. On Day 0, ldlA and ldlA[haSR-BI] cells were plated (2.5×10^6 cells/well in six-well dishes) in medium A or B, respectively, and the assay was performed on day 2. Transfected COS cells were prepared as described above. Binding assays were performed as described above, with the following minor modifications. Cells were prechilled on ice for 30 min., incubated with the indicated radiolabeled ligands (^{125}I-LDL, ^{125}I-AcLDL, or ^{3}H-labeled liposomes) in ice-cold medium D (Ham's F-12 containing 0.5% (w/v) fatty acid free bovine serum albumin (FAF-BSA) and 10 mM HEPES, pH 7.4), with or without unlabeled competitors, for 2 h at 4°C with gentle shaking. Cells were then washed twice with Tris wash buffer (60 mM Tris-HCl, 0.15 M NaCl, pH 7.4) containing 2 mg/ml FAF-BSA, followed by one rapid wash with Tris wash buffer without FAF-BSA. The cells were then solubilized with 0.1 N NaCl, and radioactivity and protein determinations were made as described above. The specific, high affinity ligand binding activities shown represent the differences between values obtained in the absence (total binding) and presence (nonspecific binding) of an excess of the indicated unlabeled ligands. Nonspecific binding of [^{3}H]PS liposomes to cells were generally low. The binding values are expressed as nanograms of bound ^{125}I-labeled protein or ng of total phospholipids from ^{3}H-labeled liposomes/milligram of cell protein.

Other procedures.

Protein concentrations were determined by the method of Lowry et al. 1951 J. Biol. Chem. 193, 265-275. DNA sequencing was performed using Sequenase 2.0 kit according to manufacturers instructions and the reported results for the coding region were verified by determining the sequence from both strands of the template. Oligonucleotide primers were prepared in the MIT Biopolymers Laboratory. Polymerase chain reaction (PCR) was used to generate a fragment of the hamster class A scavenger receptor DNA as follows. Primers based on the sequence of one exon of the murine scavenger receptor gene (Ashkenas et al., 1993 J. Lipid Res. 34, 983-1000) (5' AATGAAGAACTGCTTAGTTT 3' (Sequence ID No. 1) and 5' AATCAAGGAATTTAACTG 3' (Sequence ID No. 2)) were used to amplify a fragment of the expected size (240 base pairs) from hamster ldlA cell genomic DNA. This amplicon was cloned into pCR1000 (Invitrogen) to generate the plasmid pSA1, which was sequenced.

Results

Isolation and Characterization of variant Var-261

High affinity binding, uptake and degradation of AcLDL

The LDL receptor-deficient CHO cell line ldlA was processed and subjected to nutritional selection for rare variants which expressed endocytic receptors for AcLDL. One of the isolates, designated Var-261, was examined further. Var-261 and ldlA cells were plated on day 0 at 350,000 and 250,000 cells/well, respectively, in 6-well dishes in medium B plus 3% (v/v) newborn calf lipoprotein-deficient serum, and assayed on day 2 as described above. ^{125}I-AcLDL binding at 4°C for 2 hrs was measured using method B. ^{125}I-AcLDL binding plus uptake and degradation at 37°C was measured after a 5 hr incubation. The high affinity values shown represent the differences between measurements made in the absence (duplicate incubations) and presence (single incubations) of an excess of the unlabeled competitor M-BSA (100 μg/ml).

As shown by Figure 2A, these cells exhibited high affinity, saturable ^{125}I-AcLDL binding at 4°C. The binding at 37°C and subsequent uptake shown in Figure 2B and lysosomal degradation shown in Figure 2C were characteristic of receptor-mediated endocytosis. Degradation, but not binding plus uptake, at 37°C was inhibited by the lysosomotrophic drug chloroquine. In contrast, there was virtually no high affinity binding, uptake, or degradation of ^{125}I-AcLDL by the parental ldlA cells or wild-type CHO cells. The scavenger receptor activity in Var-261 cells was not suppressed by the addition of sterols to the medium.

Broad polyanion binding specificity

A hallmark of macrophage scavenger receptors is their broad polyanion binding specificity, usually assessed by competition with polyanionic ligands for ^{125}I-AcLDL binding, uptake and degradation. For further characterization, the ligand specificity of the scavenger receptor activity of Var-261 cells was compared with that of transfected COS cells transiently expressing the murine type II macrophage scavenger receptor, a class A scavenger receptor (mSR-AII).

COS cells were transfected on day 1 with murine scavenger receptor type II. On day 2 the transfected COS cells and Var-261 cells were set in 6-well dishes (1×10^6 and 0.5×10^6 cells/well, respectively). On day 3, ^{125}I-AcLDL (5 μg/ml) in medium B plus 10% fetal bovine serum was added to the cells and degradation was measured after a 5 hr incubation at 37°C in the absence (duplicate determinations) or presence (duplicate determinations) of the indicated amounts of polyanions. The 100% of control values (ng/5 hr/mg cell protein) from the two experiments were: Var-261, 606 and 473; and COS[mSR-AII], 1982 and 976. For the Var-261 and COS[mSR-AII] cells, the 100% of control values were: 321 and 390; and 848 and 1014, respectively.

The classic scavenger receptor polyanionic ligands AcLDL, fucoidin, and poly G were all effective competitors of ^{125}I-AcLDL degradation by both Var-261 and the transfected COS cells, and native LDL did not inhibit the activity in either cell type. Nevertheless, the binding specificity in Var-261 cells differed from that in macrophages and for class A receptors in several critical ways. Poly I, a very effective competitor of class A scavenger receptors (greater than 50% inhibition at 2.5 μg/ml) only partially inhibited the activity in Var-261 cells (34% inhibition at 400 μg/ml). In contrast, maleylated BSA (M-BSA) inhibited the scavenger receptor activity in Var-261 cells at concentrations far lower that those required for inhibition of the murine type II receptors. The ReLPS form of endotoxin, which is a scavenger receptor competitor exhibiting complex binding properties for class A scavenger receptors (Ashkenas et al., 1993), only partly inhibited the activity in Var-261 cells at the high concentration of 250 μg/ml.

Determination if hamster scavenger receptor DNA is expressed in Var-261 cells

The striking differences in ligand specificities between the scavenger receptor activity in Var-261 cells and those exhibited by the murine and bovine class A scavenger receptors (Ashkenas et al., 1993; Kodama et al., 1990; Rohrer et al., 1990) strongly suggested that the Var-261 cells did not express class A receptors. However, there was a possibility that the novel specificity was merely a consequence of species differences (e.g., hamster-derived Var-261 cells vs. murine SR-AII). To determine if this was the case, a 240 base pair fragment of the hamster class A macrophage scavenger receptor gene was isolated by polymerase chain reaction (PCR) using genomic CHO cell DNA as a template. This hamster gene fragment, which is found in both type I and II macrophage scavenger receptors, was cloned and sequenced. This fragment has between 75%-85%

nucleotide sequence identity with its human, murine, rabbit and bovine counterparts (Kodama et al., 1990; Rohrer et al., 1990; Ashkenas et al., 1993; Matsumoto et al., 1990 Proc. Natl. Acad. Sci. USA 87, 9133-9137; Bickel and Freeman, 1992) and, with the exception of the amino acid corresponding to murine position 117, was consistent with the previously identified consensus sequence defined for residues 95 - 159 (Ashkenas et al, 1993).

This hamster fragment was used as a probe in Northern analysis of Var-261 cell mRNA. Under conditions where the hamster probe recognized murine scavenger receptor mRNA from CHO cells transfected with the murine scavenger receptor cDNA (Ashkenas et al., 1993), no signal was detected in the hamster-derived Var-261 or control CHO cell mRNA, even after extensive overexposure of the film. Thus, Var-261 cells do not express significant amounts of the hamster type I or II class A receptors and must instead express a different scavenger receptor.

Cloning of haSR-BI receptor cDNA from Var-261

In an attempt to clone a cDNA encoding this apparently new scavenger receptor, a cDNA expression library was prepared from Var-261 poly A^+ mRNA, the library was divided into small pools (approximately 5000 clones/pool), the pools were transfected into COS cells and the transiently transfected cells visually screened for endocytosis of fluorescent AcLDL (DiI-AcLDL). A receptor-positive pool was obtained after screening approximately 450,000 clones and this pool was subdivided repeatedly until a single functional plasmid (designated phaSR-BI for plasmid encoding hamster scavenger receptor type BI) was obtained.

Figures 3A and 3B are graphs of ^{125}I-AcLDL binding to haSR-BI expressed in transfected COS cells: Figure 3A demonstrates the concentration dependence in COS[haSR-BI] cells and Figure 3B demonstrates the ligand specificity in ldlA[haSR-BI] cells. To measure

binding of AcLDL, on day 1, COS cells were transfected with the expression vector for the hamster scavenger receptor type BI (haSR-BI). On day 2 the transfected COS cells were plated in 6-well dishes (1×10^6 cells/well in medium A plus 1 mM sodium butyrate). On day 3 between 0 and 50 μg/ml of ^{125}I-AcLDL were added to the monolayers at 4°C and binding was measured using Method A. The high affinity values shown in the Figures represent the differences between measurements made in the absence (duplicate incubations) and presence (single incubations) of an excess of the unlabeled competitor M-BSA (200 μg/ml). To measure binding specificity, on day 1, Var-261 and ldlA[haSR-BI] cells were plated in 6-well dishes (0.2×10^6 cells/well in MAC medium or medium B containing 10% fetal bovine serum and 0.25 mg/ml G418, respectively). On day 3 the binding of ^{125}I-AcLDL (5 μg/ml) at 4°C in the absence or presence of the indicated competitors was measured using Method A. The values shown in the Figures represent the averages of duplicate determinations. The 100% of control values for Var-261 and ldlA[haSR-BI] were 1809 and 293 ng/mg cell protein, respectively.

The HaSR-BI plasmid conferred high affinity (K_d(4°C) approximately 5 μg protein/ml) ^{125}I-AcLDL binding on transiently transfected COS cells, as shown by Figure 3A. The ligand specificity of haSR-BI expressed in stably transfected ldlA cells was compared to that of the Var-261 cells, as shown in Figure 3B. M-BSA inhibited the binding of ^{125}I-AcLDL to cells expressing haSR-BI and to Var-261 cells. Unexpectedly, haSR-BI was not inhibited by poly G, fucoidin or carrageenan (another classic scavenger receptor ligand (Brown and Goldstein, 1983)), which were competitors for the activity in Var-261 cells. In addition, LDL did not inhibit the binding of ^{125}I-AcLDL to Var-261 cells, but was a competitor for cells expressing haSR-BI. There is no other case in which native LDL has been reported to block scavenger

receptor activity. Thus, the specificity of the haSR-BI differs dramatically from that reported for any previously described scavenger receptor, including that in Var-261 cells. Since the ligand specificities of the class B receptors described in more detail below are very different from the activity in Var-261 cells, it seems unlikely that either of these two class B scavenger receptors accounts for the binding activity observed in the Var-261 cells.

Northern blot analysis using haSR-BI as a probe showed that a single major mRNA (approximately 3.4 kb) was present in similar amounts in both the parental ldlA and Var-261 cells, indicating that the cells express similar amounts of haSR-BI mRNA. Therefore, although haSR-BI encodes a scavenger receptor, it is apparently expressed in both ldlA and Var-261 cells at very low levels. Taken together with the specificity data described above, these results indicate that haSR-BI is a novel scavenger receptor which was not responsible for most of the scavenger receptor activity in Var-261 cells.

Analysis of haSR-BI cDNA

The cloned haSR-BI cDNA is approximately 2.9 kb long. The sequences of the 5′ untranslated region, the coding region, and a portion of the 3′ untranslated region are shown in Sequence Listing ID No. 3. The predicted protein sequence is 509 amino acids (Sequence Listing ID No. 4) with a calculated molecular weight of 57 kD. The murine cDNA was subsequently isolated. The nucleotide sequence is shown in Sequence Listing ID No. 7 and the predicted amino acid sequence is shown in Sequence Listing ID No. 8.

Based on comparison of amino acid sequence, haSR-BI has homology along its entire length to members of the CD36 family of membrane proteins: CD36 (32%, 31% and 33% amino acid identities with the human (Oquendo et al., 1989), murine (Endemann et al., 1993) and rat (also known as "FAT", Abumrad et al., 1993) homologs),

rat LIMPII (33%, a lysosomal integral membrane protein (Vega et al., 1991 J. Biol. Chem. 266, 16818-16824), and two *Drosophila melanogaster* proteins, *emp* (29%, Hart and Wilcox, 1993 J. Mol. Biol. 234, 249-253) and "dCD36" (31%, Genbank #DMCD361). All of these, with the exception of "dCD36", have two internal hydrophobic domains, which have been suggested to serve as membrane spanning domains, and a series of conserved cysteines and putative N-linked glycosylation sites (see Vega et al., 1991 for a description of the common sequence elements).

Characterization and comparison of haSR-BI binding with human CD36 binding affinity and selectivity

To further characterize the binding properties of huCD36 and compare them with those of haSR-BI, the properties of COS cells transiently transfected with a cDNA expression vector for human CD36 (obtained from Dr. Brian Seed, Oquendo et al., 1989) were examined.

COS cells were transfected on day 1 with a plasmid encoding CD36. On day 2 the transfected cells were set in 6-well dishes at 1 x 10^6 cells/well in medium A plus 1 mM sodium butyrate. On day 3, ^{125}I-AcLDL binding at 4°C was measured. The high affinity values shown represent the differences between measurements made in the absence (duplicate incubations) and presence (single incubations) of an excess of the unlabeled competitor M-BSA (200 μg/ml). On day 3, degradation of ^{125}I-AcLDL (5 μg/ml) was measured after a 5 hr incubation at 37°C in the absence (duplicate determinations) or presence (duplicate determinations) of the indicated competitors. The values represent the means of four determinations from two identical experiments (the error bars represent standard deviations). The 100% of control values (ng/5 hr/mg cell protein) from the two experiments were 232 and 103.

Figure 4A shows that expression of huCD36 conferred high affinity ^{125}I-AcLDL binding on transiently transfected COS cells at 4°C with an

apparent dissociation constant in the range of approximately 5 μg protein/ml. Figure 4B shows that the receptor activity was inhibited by M-BSA and AcLDL, but not by other polyanions which inhibit class A macrophage scavenger receptors, including fucoidin, poly I, poly G, and ReLPS. Figure 5 is a graph directly comparing lipoprotein inhibition of ^{125}I-AcLDL binding to haSR-BI and huCD36. The graph clearly demonstrates the similarities: i.e., binding to both receptors is inhibited by the modified LDL; and the differences: only haSR-BI binding is inhibited by LDL.

HDL Binding to haSR-BI

Further binding studies were conducted to determine if SR-BI bound HDL and VLDL, as well as LDL and anionic phospholipids. Competition binding studies demonstrate that HDL and VLDL (400 μg/ml) competitively inhibit binding of ^{125}I-AcLDL to HaSR-BI, providing further support for the potential role of this receptor in lipoprotein and lipid metabolism. Direct binding of ^{125}I-HDL to cells expressing SR-BI is shown in Figure 8a. Studies were also conducted using CD36, which demonstrated that HDL bound competitively to CD36.

P*hospholipid Binding*

To determine if phospholipids could bind to haSR-BI, 105 nm diameter PS liposomes (PS/phosphatidylcholine/cholesterol, ratio 1:1:1) radiolabeled with trace amounts of [^{3}H]dipalmitoyl phosphatidylcholine (62 Ci/mmol) were prepared and binding at 4∘C to untransfected cells (ldlA) and transfected cells which express haSR-Bl (ldlA[haSR-BI] cells determined. The results are shown in Figures 7a, 7b, 7c, 7d, and 7e. Fig 7d shows that there was substantial, high affinity (K_d-15 μg phospholipid/ml) and saturable binding to the transfected cells, but relatively little binding to the untransfected cells. Assuming that the phospholipid and cholesterol were uniformly distributed in homogenous liposomes

containing approximately 107,400 molecules of phospholipid/liposome, it is estimated that the K_d (mol of PS liposomes/liter) to be approximately 0.18 nM. PS binding was apparently independent of divalent cations because it was not inhibited by EDTA (1-10 mM). Binding depended on the phospholipid composition of the liposomes. In contrast to that of $[^3H]$PS liposomes, the binding of radiolabeled PC liposomes (PC/cholesterol, 2:1) was very low and similar to $[^3H]$PS binding to untransfected ldlA cells.

These results indicated that phospholipids can bind to haSR-BI and that this binding might depend on the charge of the phospholipid head group. The specificity of the binding was further assessed by determining the competition for $[^3H]$PS binding by unlabeled liposomes of various compositions (indicated phospholipid/PC/cholesterol, ratio 1:1:1). Figure 7e shows that the anionic phospholipids PS and PI were effective inhibitors while the zwitterionic PC and PE as well as SM were not. PA, another anionic phospholipid, was able to compete, but not as effectively as PS and PI. Figure 7a shows that PS and PI liposomes inhibited virtually all of the binding of ^{125}I-AcLDL to haSR-BI in transiently transfected COS cells (greater than 50% inhibition at concentrations greater than 10 μg/phospholipid/ml), while PC had virtually no effect at concentrations as high as 250 μg phospholipid/ml. Similar results are shown for cells transfected with huCD36 in Figure 7b. The extent of PS inhibition of ^{125}I-LDL binding depended on the relative PS content of the liposomes. Figure 7c shows that inhibition by 500 μg phospholipid/ml increased substantially as the amount of PS in PS/PC mixed liposomes increased from 0 to 50 mol % of total phospholipid, with greater than 50% inhibition occurring when the PS mol % was greater than 10. These competition experiments suggest that anionic phospholipids bound to haSR-BI at a site close to or identical with the site of native and modified LDL

binding and that polyvalent binding via multiple anionic phospholipid molecules may be involved.

The specific recognition of anionic phospholipids in the outer leaflets of cell membranes and lipoproteins by cell surface receptors may play an important role in a variety of physiologic and pathophysiologic process, including recognition of damaged or senescent cells by the reticuloendothelial system or lipoprotein hemeostasis. These studies support the role of SR-BI in these interactions.

Tissue distribution of haSR-BI

To explore the physiological functions of haSR-BI, the tissue distribution of haSR-BI was determined in murine tissues and during differentiation of 3T3-L1 cells into adipocytes using Northern blot. Each lane was loaded with 0.5 μg of poly(A)+ RNA prepared from the murine tissues: kidney, liver, brain, testis, fat, diaphragm, heart, lung, spleen, or from 3T3-L1 fibroblasts which were either nonconfluent at the fibroblast stage or confluent and induced to differentiate over a period of 0, 2, 4, 6 or 8 days. The blots were hybridized with a 600 base pair fragment of the coding region of haSR-BI. Hsp70 cDNA was used as a control for equal RNA loading. In the Northern of the murine tissues, the same blot was used for both SR-BI and hsp70 hybridizations. In the Northern of the differentiating 3T3-L1 cells, parallel blots were used. The blot of the murine tissues was also hybridized with a CD36 probe demonstrating that CD36 and SR-BI probes recognized different mRNA species.

One predominant band of approximately 2.4 kb was most abundant in fat and was present at moderate levels in lung and liver. There was little expression in the remaining tissues tested, which included kidney, brain, testis, diaphragm, heart, and spleen. To further investigate the expression of SR-BI in fat, the SR-BI mRNA levels in 3T3-L1 cells which were induced to differentiate into adipocytes were

determined. The levels of SR-BI mRNA were found to increase during differentiation in a manner similar to that previously demonstrated for the glucose transporter GLUT4 and for Rab3D, a small molecular weight GTP binding protein (Baldini et al., 1992). These data, together with the data showing that modified LDL binding is inhibited by LDL, indicate that SR-BI plays a physiological role in lipid metabolism in adipocytes. In this regard, it is noteworthy that rat CD36 (also known as the "FAT" protein) was cloned as a result of its ability to directly bind reactive fatty acid esters (Abumrad et al., 1993). CD36 message is also markedly increased upon differentiation of the cultured lines 3T3 F442A and Ob1771 into adipocytes, as is the message for SR-BI in the 3T3-L1 adipocyte system. The expression patterns of SR-BI mRNA and CD36 mRNA are similar, but not identical. Both are found in high levels in adipose tissue. One notable difference in expression levels is found in the liver, where SR-BI expression is moderate but no CD36 message was detected. The observation that native LDL competes for AcLDL binding to SR-BI further indicated that SR-BI may play a role in lipid metabolism.

In contrast to the studies detecting mRNA encoding SR-BI, blots using polyclonal antibodies to a cytoplasmic region of SR-BI found that very high levels of protein were present in liver, adrenal tissues, and ovary in mice and rats, but only very low or undetectable levels in either white or brown fat, muscle or a variety of other tissues. Bands in the rat tissues were present at approximately 80 to 95 kD in liver but a smaller molecular weight protein of approximately 57 to 69 kD was detected in the steroidogenic tissues, including adrenal tissues and testes. This indicates that the mRNA present in the adipose or steroidogenic tissue actually encodes a close relative of SR-BI, rather than SR-BI, that the SR-BI mRNa is not translated into protein in fat in

rodents, and/or that there may be alternative splicing of the SR-BI gene. In the mouse tissues, only the 80 to 95 kD form was observed in the liver and steroidogenic tissues. This is the same form observed in transfected cultured cells.

Recycling of HDL and Lipid Uptake Studies

It is significant that SR-BI binds HDL and is present in high levels in the liver and in tissues which are known to take up cholesterol from HDL for use in synthesis of steroids. Further studies were conducted to determine if the HDL bound to cells expressing SR-BI is recycled, i.e., if lipid bound to the HDL is taken up by the cell over time while the amount of HDL remains relatively constant. This is demonstrated in Figure 8b. It is evident that the amount of HDL bound by the cells, as measured using ^{125}I-labeled HDL, remains relatively constant after a steady state condition is reached thirty minutes to an hour after addition of the HDL to the cells. In contrast, the amount of fluorescent lipid in the cells continues to increase over time, demonstrating that the HDL must be binding the cells, not being internalized or degraded, and that the lipid bound to the HDL being internalized and retained within the cells. The results with DiI are predictive of results with cholesterol ester. Controls with untransfected cells, in which there was no binding to the cells or fluorescent lipid transfer, are evidence that this is a receptor dependent function.

The possible roles of SR-BI in HDL metabolism is shown schematically in Figure 9. It is extremely likely that SR-BI and the related SR-B proteins play critical roles in HDL mediated lipid metabolism and transport. SR-BI appears to be responsible for cholesterol delivery to steroidogenic tissues and liver. It would be useful to increase expression of SR-BI in cells in which uptake of cholesterol can be increased, freeing HDL to serve as a means for removal

of cholesterol from storage cells such as foam cells where it can play a role in atherogenesis.

***II. Drosophila melanogaster* scavenger receptor protein**

In an effort to further define the structures and functions of scavenger receptors, receptor expression was investigated in a representative invertebrate, *Drosophila melanogaster* (Abrams, et al. 1993 Proc. Natl. Acad. USA 89:10375-10379). By examining *D. melanogaster* embryos using fluorescently labeled AcLDL and ^{125}I-AcLDL as probes for receptor activity *in vivo* and *in vitro*, fluorescence was found to be distributed throughout the interstitial spaces of the body cavity in a pattern characteristic of the distribution of embryonic macrophages and was observed in cells with multivesicular inclusions characteristic of macrophages. Further analysis of primary embryonic cell cultures showed that uptake was macrophage specific and exhibited the broad specificity of mammalian scavenger receptors.

Two commonly used *Drosophila* cell lines were examined for scavenger receptor activity: L2 and Kc. Only the Schneider L2 cells, not the Kc cells, exhibit a scavenger receptor-mediated endocytic pathway, which is almost identical to that of mammalian macrophages. The L2 cell receptors exhibit characteristic scavenger receptor-like broad polyanion-binding specificity, and mediate high-affinity and saturable binding, uptake and degradation of AcLDL. In L2 cells, the kinetics of intracellular ligand degradation after binding and uptake shows a lag phase, intracellular ligand degradation is chloroquine sensitive, and endocytosis is temperature dependent.

Preparation of cDNA and genomic libraries:

To facilitate both hybridization and expression cloning, cDNA libraries were generated from poly A+ RNA from L2 cells using standard procedures, as described above for Var-261 cells, and libraries were generated in the expression vector pcDNA1

(Invitrogen). The average insert size for the bidirectional L2 cell library is about 1.4 kb. DNAs for hybridization and transfection experiments were prepared as follows: *E. coli* strain MC1061/P3 is transformed with the cDNA expression library by electroporation and the transformed cells plated on LB-A/T plates (LB with Amp (15 μg/ml)/Tet (8 μg/ml)) to obtain isolated colonies at densities of 2,000 to 10,000 per 150 mm dish. Each plate, representing one pool, is scraped to recover the bacteria and a pool of library plasmids is isolated from each dish of bacteria using a midiprep method (miniprep method of Sambrook et al., 1989 Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, scaled up five to ten fold). The pools can be used for Southern blot analysis for hybridization screening and for transfection into COS or CHO cells for expression cloning.

Expression in cultured mammalian cells.

Expression of the receptors can be detected using either or both fluorescence microscopy and light microscopy of emulsion autoradiographs. Transient expression can be routinely obtained in COS cells, stable expression in CHO cells.

Fluorescence screening: Scavenger receptor activity can be detected using fluorescent, 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate-labeled AcLDL (DiI-AcLDL). On day 0, COS M6 cells are plated in 35 mm culture dishes (3 to 4 x 10^5 cells/dish) in medium A (DMEM supplemented with 100 units penicillin/ml medium, 100 μg streptomycin/ml medium, and 2 mM glutamine) containing 10% FBS (medium B). On day 1, the cells are transfected with 0.5 μg/dish of library DNA following the method of Cullen, 1987 Methods in Enz. 152:684-704. On day 2, monolayers were re-fed with modified medium B 1 mM sodium butyrate. On day 3, the monolayers are re-fed with modified medium B containing between one and five μg protein/ml of DiI-AcLDL. After a 5 hr incubation

at 37°C, the plates are washed two times with PBS and the cells fixed with 3.7% formaldehyde in PBS for 10 min at room temperature. The presence of fluorescent DiI in the fixed cells is determined by visual screening using a Lietz inverted fluorescence microscope.

After screening the bidirectional L2 cell library, a pool of approximately 3500 clones which reproducibly conferred DiI-AcLDL endocytic activity in transiently transfected COS cells was identified. This pool was subdivided into 18 subpools of approximately 350 clones each, which were transfected into COS cells. One of these subpools also conferred DiI-AcLDL endocytic activity.

The clone from the subpool was isolated and transfected into CHO cells to yield CHO[dSR-CI]-2.6a cells. The nucleic acid (Sequence ID No. 5) and amino acid (Sequence ID No. 6) sequences were also obtained using the methods described above.

<u>Binding Activity of CHO[dSR-CI]-2.6a Cells</u>

Binding of ^{125}I-AcLDL to CHO[dSR-CI]-2.6a cells was measured as ng AcLDL protein bound/mg cell protein at concentrations of 1, 3, 6, 13, 25, and 50 μg AcLDL protein/ml. Uptake was also measured, comparing total, nonspecific and specific binding.

As shown by Figure 6A, dSR-CI binds AcLDL. As shown by Figure 6B, AcLDL was also taken up by the cells expressing dSR-CI.

Degradation was measured to confirm that dSR-CI mediated internalization of the AcLDL. The results shown in Figure 6C confirm the selective degradation of AcLDL.

Competitive binding of AcLDL was performed to more accurately define the specificity of the AcLDL binding. The results are shown below in Table 1.

Table 1. Competitive Inhibition of ^{125}I-AcLDL Degradation Activity by CHO[dSR-CI]-2.6a Cells.

Competitor	Degradation (% No Competitor)
None	100
AcLDL (400 μg/ml)	4.8
LDL (400 μg/ml)	97.6
M-BSA (400 μg/ml)	6.7
BSA (400 μg/ml)	94.4
poly I (400 μg/ml)	2.7
dA5G37 (100 μg/ml)	5.1
dA37 (100 μg/ml)	98.2
dA37 (400 μg/ml)	43.0
Dextran Sulfate	4.2
Dextran (400 μg/ml)	100
Poly D-glutamic acid (400 μg/ml)	34

III. Applications of the Scavenger Receptor Proteins

The presence of scavenger receptors on both mammalian and *Drosophila* macrophages suggests that they mediate critical, well-conserved functions, possibly pathogen recognition, and raises the possibility that they may have appeared early in the evolution of host defense systems. In this regard, it is known that postembryonic macrophage-like hemocytes in *Drosophila* participate in wound healing, encapsulation of pathogens, and phagocytosis. Furthermore, macrophages play an important role in the recognition of apoptotic or senescent cells during the course of development, normal cell turnover, and aging, although it is not known if the scavenger receptors are also involved in these processes. The binding specificity of the SR-BI protein implicates this receptor in additional or alternative roles critical to HDL metabolism and the delivery of cholesterol to steroidogenic tissues.

Accordingly, the understanding of the structure and functions of the receptor proteins described herein, as well as the cDNAs encoding these proteins, have a variety of uses. Specifically, the proteins and their DNAs can be used in screening of drugs which modulate the activity and/or the expression of the receptors; in screening of patient samples for the

presence of functional receptor protein; in the case of the SR-BI receptor protein, removal of LDL, modified LDL, VLDL, or HDL by reaction with immobilized receptor protein; use of the DNA to construct probes for screening of libraries for other receptors, including the human equivalents, and the regulatory sequences controlling the expression of the other receptors as well as SR-BI and SR-CI. These drugs, when identified, may be useful in treating or preventing atherosclerosis, fat uptake by adipocytes, and some types of immune disorders.

<u>Isolation of other receptor proteins.</u>

The nucleotide sequences identified herein as encoding hamster SR-BI and *Drosophila melanogaster* SR-CI are useful as probes for screening of libraries for the presence of related receptors. Libraries are constructed from cells of a desired species, such as humans, which are then screened with all or a portion of the nucleotide sequence encoding either SR-BI or SR-CI. Specific regions of interest are those portions of the nucleotide sequence which encode regions of the protein conserved between different receptors; between the same receptors from different species; and within discrete regions of the receptor proteins: the cytoplasmic region, the transmembrane region, the "stem" regions that may include EGF repeats, collagen like regions α-helical coiled regions, or regions having a high density of cysteines (CCP domains), and specific ligand regions. These regions are identified by structural analysis such as that which has been used to generate the schematics in Figures 1A and 1B, using methods routinely available to those skilled in the art. These methods include chemical crosslinking, electrophoretic analysis, hydrodynamic studies, and electron microscopy and computer assisted analysis of structure based on predicted amino acid sequence.

As used herein, unless specifically stated otherwise, the term "SR-BI" refers to the nucleotide

and amino acid sequences, respectively, shown in Sequence ID Nos. 3 and 4, and 7 and 8, and degenerate variants thereof and their equivalents in other species of origin, especially human, as well as functionally equivalent variants, having additions, deletions, and substitutions of either nucleotides or amino acids which do not significantly alter the functional activity of the protein as a receptor characterized by the binding activity identified above. The term "SR-CI" refers to the sequences shown in Sequence ID Nos. 5 and 6, and degenerate variants thereof and their equivalents in other species of origin, especially human, as well as functionally equivalent variants, having additions, deletions, and substitutions of either nucleotides or amino acids which do not significantly alter the functional activity of the protein as a receptor characterized by the binding activity identified above.

Preferred uses for these sequences, especially those in the Sequence Listings below, are for the cloning of equivalent receptor molecules present in human cells, for the isolation and characterization of the regulatory sequences present in the genome which controlled the extent to which a particular receptor is expressed in a cell, and for the screening of drugs altering binding of or endocytosis of ligand by the scavenger receptor proteins.

Isolation of Receptor Proteins

Additional receptor proteins for study can be obtained by expression in suitable recombinant host systems, such as mammalian, yeast, bacteria, or insect cells. Isolation can be facilitated by making antibodies to the recombinant protein which are then immobilized on substrates for use in purification of additional receptors.

As discussed above with regard to tissue distribution, it appears that at least one different but closely related protein is encoded by a nucleotide sequence present in steroidogenic tissues in rodents

which hybridizes to Sequence ID No. 3 under stringent conditions, and can be isolated using routine techniques and the materials described herein.

Screening of patient samples for expression of receptor proteins.

The sequences disclosed herein are useful in screening of patient samples for the presence of normal receptor proteins, using hybridization assays of patient samples, including blood and tissues. Screening can also be accomplished using antibodies, typically labeled with a fluorescent, radiolabeled, or enzymatic label, or by isolation of target cells and screening for binding activity, as described in the examples above. Typically, one would be screening for expression on either a qualitative or quantitative basis, and for expression of functional receptor.

Hybridization Probes

Reaction conditions for hybridization of an oligonucleotide probe or primer to a nucleic acid sequence vary from oligonucleotide to oligonucleotide, depending on factors such as oligonucleotide length, the number of G and C nucleotides, and the composition of the buffer utilized in the hybridization reaction. Moderately stringent hybridization conditions are generally understood by those skilled in the art as conditions approximately 25°C below the melting temperature of a perfectly base-paired double-stranded DNA. Higher specificity is generally achieved by employing incubation conditions having higher temperatures, in other words more stringent conditions. In general, the longer the sequence or higher the G and C content, the higher the temperature and/or salt concentration required. Chapter 11 of the well-known laboratory manual of Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, second edition, Cold Spring Harbor Laboratory Press, New York (1990) (which is incorporated by reference herein), describes hybridization conditions for oligonucleotide probes and primers in great detail, including a description

of the factors involved and the level of stringency necessary to guarantee hybridization with specificity.

The preferred size of a hybridization probe is from 10 nucleotides to 100,000 nucleotides in length. Below 10 nucleotides, hybridized systems are not stable and will begin to denature above 20°C. Above 100,000 nucleotides, one finds that hybridization (renaturation) becomes a much slower and incomplete process, as described in greater detail in the text MOLECULAR GENETICS, Stent, G.S. and R. Calender, pp. 213-219 (1971). Ideally, the probe should be from 20 to 10,000 nucleotides. Smaller nucleotide sequences (20-100) lend themselves to production by automated organic synthetic techniques. Sequences from 100-10,000 nucleotides can be obtained from appropriate restriction endonuclease treatments. The labeling of the smaller probes with the relatively bulky chemiluminescent moieties may in some cases interfere with the hybridization process.

Generation of Antibodies for Diagnostic or Therapeutic Use

Antibodies to the receptor proteins can also be generated which are useful in detection, characterization or isolation of receptor proteins, as well as for modifying receptor protein activity, in most cases, through inhibition of binding. Antibodies are generated by standard techniques, using human or animal receptor proteins. Since the proteins exhibit high evolutionary conservation, it may be advantageous to generate antibodies to a protein of a different species of origin than the species in which the antibodies are to be tested or utilized, looking for those antibodies which are immunoreactive with the most evolutionarily conserved regions. Antibodies are typically generated by immunization of an animal using an adjuvant such as Freund's adjuvant in combination with an immunogenic amount of the protein administered over a period of weeks in two to three week intervals, then isolated from the serum, or used to make hybridomas which express the antibodies in culture.

Because the methods for immunizing animals yield antibody which is not of human origin, the antibodies could elicit an adverse effect if administered to humans. Methods for "humanizing" antibodies, or generating less immunogenic fragments of non-human antibodies, are well known. A humanized antibody is one in which only the antigen-recognized sites, or complementarily-determining hypervariable regions (CDRs) are of non-human origin, whereas all framework regions (FR) of variable domains are products of human genes. These "humanized" antibodies present a lesser xenographic rejection stimulus when introduced to a human recipient.

To accomplish humanization of a selected mouse monoclonal antibody, the CDR grafting method described by Daugherty, et al., 1991 Nucl. Acids Res., 19:2471-2476, incorporated herein by reference, may be used. Briefly, the variable region DNA of a selected animal recombinant anti-idiotypic ScFv is sequenced by the method of Clackson, T., et al., 1991 Nature, 352:624-688, incorporated herein by reference. Using this sequence, animal CDRs are distinguished from animal framework regions (FR) based on locations of the CDRs in known sequences of animal variable genes. Kabat, H.A., *et al.*, Sequences of Proteins of Immunological Interest, 4th Ed. (U.S. Dept. Health and Human Services, Bethesda, MD, 1987). Once the animal CDRs and FR are identified, the CDRs are grafted onto human heavy chain variable region framework by the use of synthetic oligonucleotides and polymerase chain reaction (PCR) recombination. Codons for the animal heavy chain CDRs, as well as the available human heavy chain variable region framework, are built in four (each 100 bases long) oligonucleotides. Using PCR, a grafted DNA sequence of 400 bases is formed that encodes for the recombinant animal CDR/human heavy chain FR protection.

The immunogenic stimulus presented by the monoclonal antibodies so produced may be further

decreased by the use of Pharmacia's (Pharmacia LKB Biotechnology, Sweden) "Recombinant Phage Antibody System" (RPAS), which generates a single-chain Fv fragment (ScFv) which incorporates the complete antigen-binding domain of the antibody. In the RPAS, antibody variable heavy and light chain genes are separately amplified from the hybridoma mRNA and cloned into an expression vector. The heavy and light chain domains are co-expressed on the same polypeptide chain after joining with a short linker DNA which codes for a flexible peptide. This assembly generates a single-chain Fv fragment (ScFv) which incorporates the complete antigen-binding domain of the antibody. Compared to the intact monoclonal antibody, the recombinant ScFv includes a considerably lower number of epitopes, and thereby presents a much weaker immunogenic stimulus when injected into humans.

The antibodies can be formulated in standard pharmaceutical carriers for administration to patients in need thereof. These include saline, phosphate buffered saline, and other aqueous carriers, and liposomes, polymeric microspheres and other controlled release delivery devices, as are well known in the art. The antibodies can also be administered with adjuvant, such as muramyl dipeptide or other materials approved for use in humans (Freund's adjuvant can be used for administration of antibody to animals).

<u>Screening for drugs modifying or altering the extent of receptor function or expression</u>

The receptor proteins are useful as targets for compounds which turn on, or off, or otherwise regulate binding to these receptors. The assays described above clearly provide routine methodology by which a compound can be tested for an inhibitory effect on binding of a specific compound, such as a radiolabeled modified LDL or polyion. The *in vitro* studies of compounds which appear to inhibit binding selectively to the receptors are then confirmed by animal testing. Since the molecules are so highly evolutionarily conserved, it is possible to conduct studies in

laboratory animals such as mice to predict the effects in humans.

Studies based on inhibition of binding are predictive for indirect effects of alteration of receptor binding. For example, inhibition of LDL binding to the SR-BI receptor leads to decreased uptake by cells of LDL and therefore decreases deposition of LDL in cells; similar effects should be observed for inhibition of lipoprotein and/or lipid by adipocytes expressing the SR-BI receptor. Conversely, increasing LDL binding to cells increases removal of lipids from the blood stream and thereby decreases lipid deposition within the blood stream. Studies have been conducted using a stimulator to enhance macrophage uptake of cholesterol and thereby treat atherogenesis, using M-CSF (Schaub, et al., 1994 Arterioscler. Thromb. 14(1), 70-76; Inaba, et al., 1993 J. Clin. Invest. 92(2), 750-757). Although the target of the stimulator is not known with specificity, this provides further support for the rationale for believing the indirect *in vivo* effects can be achieved based on the *in vitro* binding data.

Studies described above and the results shown in Figure 8 demonstrate that drugs increasing expression of SR-BI or closely related proteins in tissues such as liver would be useful in enhancing removal of cholesterol from the circulation and foam cells.

Assays for testing compounds for useful activity can be based solely on interaction with the receptor protein, preferably expressed on the surface of transfected cells such as those described above, although proteins in solution or immobilized on inert substrates can also be utilized, where the indication is inhibition or increase in binding of LDL or modified LDL.

Alternatively, the assays can be based on interaction with the gene sequence encoding the receptor protein, preferably the regulatory sequences directing expression of the receptor protein. For

example, antisense which binds to the regulatory sequences, and/or to the protein encoding sequences can be synthesized using standard oligonucleotide synthetic chemistry. The antisense can be stabilized for pharmaceutical use using standard methodology (encapsulation in a liposome or microsphere; introduction of modified nucleotides that are resistant to degradation or groups which increase resistance to endonucleases, such as phosphorothiodates and methylation), then screened initially for alteration of receptor activity in transfected or naturally occurring cells which express the receptor, then *in vivo* in laboratory animals. Typically, the antisense would inhibit expression. However, sequences which block those sequences which "turn off" synthesis can also be targeted.

The receptor protein for study can be isolated from either naturally occurring cells or cells which have been genetically engineered to express the receptor, as described in the examples above. In the preferred embodiment, the cells would have been engineered using the intact gene.

Random generation of receptor or receptor encoding sequence binding molecules.

Molecules with a given function, catalytic or ligand-binding, can be selected for from a complex mixture of random molecules in what has been referred to as "in vitro genetics" (Szostak, TIBS 19:89, 1992). One synthesizes a large pool of molecules bearing random and defined sequences and subjects that complex mixture, for example, approximately 10^{15} individual sequences in 100 μg of a 100 nucleotide RNA, to some selection and enrichment process. For example, by repeated cycles of affinity chromatography and PCR amplification of the molecules bound to the ligand on the column, Ellington and Szostak (1990) estimated that 1 in 10^{10} RNA molecules folded in such a way as to bind a given ligand. DNA molecules with such ligand-binding behavior have been isolated (Ellington and Szostak, 1992; Bock et al, 1992).

Computer assisted drug design

Computer modeling technology allows visualization of the three-dimensional atomic structure of a selected molecule and the rational design of new compounds that will interact with the molecule. The three-dimensional construct typically depends on data from x-ray crystallographic analyses or NMR imaging of the selected molecule. The molecular dynamics require force field data. The computer graphics systems enable prediction of how a new compound will link to the target molecule and allow experimental manipulation of the structures of the compound and target molecule to perfect binding specificity. Prediction of what the molecule-compound interaction will be when small changes are made in one or both requires molecular mechanics software and computationally intensive computers, usually coupled with user-friendly, menu-driven interfaces between the molecular design program and the user.

Examples of molecular modelling systems are the CHARMm and QUANTA programs, Polygen Corporation, Waltham, MA. CHARMm performs the energy minimization and molecular dynamics functions. QUANTA performs the construction, graphic modelling and analysis of molecular structure. QUANTA allows interactive construction, modification, visualization, and analysis of the behavior of molecules with each other.

A number of articles review computer modeling of drugs interactive with specific proteins, such as Rotivinen, et al., 1988 Acta Pharmaceutica Fennica 97, 159-166; Ripka, New Scientist 54-57 (June 16, 1988); McKinaly and Rossmann, 1989 Annu. Rev. Pharmacol. Toxiciol. 29, 111-122; Perry and Davies, QSAR: Quantitative Structure-Activity Relationships in Drug Design pp. 189-193 (Alan R. Liss, Inc. 1989); Lewis and Dean, 1989 Proc. R. Soc. Lond. 236, 125-140 and 141-162; and, with respect to a model receptor for nucleic acid components, Askew, et al., 1989 J. Am. Chem. Soc. 111, 1082-1090. Other computer programs

that screen and graphically depict chemicals are available from companies such as BioDesign, Inc., Pasadena, CA., Allelix, Inc, Mississauga, Ontario, Canada, and Hypercube, Inc., Cambridge, Ontario. Although these are primarily designed for application to drugs specific to particular proteins, they can be adapted to design of drugs specific to regions of DNA or RNA, once that region is identified.

Although described above with reference to design and generation of compounds which could alter binding, one could also screen libraries of known compounds, including natural products or synthetic chemicals, and biologically active materials, including proteins, for compounds which are inhibitors or activators.

Generation of nucleic acid regulators

Nucleic acid molecules containing the 5′ regulatory sequences of the receptor genes can be used to regulate or inhibit gene expression *in vivo*. Vectors, including both plasmid and eukaryotic viral vectors, may be used to express a particular recombinant 5′ flanking region-gene construct in cells depending on the preference and judgment of the skilled practitioner (see, e.g., Sambrook et al., Chapter 16). Furthermore, a number of viral and nonviral vectors are being developed that enable the introduction of nucleic acid sequences *in vivo* (see, e.g., Mulligan, 1993 Science, 260, 926-932; United States Patent No. 4,980,286; United States Patent No. 4,868,116; incorporated herein by reference). Recently, a delivery system was developed in which nucleic acid is encapsulated in cationic liposomes which can be injected intravenously into a mammal. This system has been used to introduce DNA into the cells of multiple tissues of adult mice, including endothelium and bone marrow (see, e.g., Zhu et al., 1993 Science 261, 209-211; incorporated herein by reference).

The 5′ flanking sequences of the receptor gene can also be used to inhibit the expression of the

receptor. For example, an antisense RNA of all or a portion of the 5' flanking region of the receptor gene can be used to inhibit expression of the receptor *in vivo*. Expression vectors (e.g., retroviral expression vectors) are already available in the art which can be used to generate an antisense RNA of a selected DNA sequence which is expressed in a cell (see, e.g., U.S. Patent No. 4,868,116; U.S. Patent No. 4,980,286). Accordingly, DNA containing all or a portion of the sequence of the 5' flanking region of the receptor gene can be inserted into an appropriate expression vector so that upon passage into the cell, the transcription of the inserted DNA yields an antisense RNA that is complementary to the mRNA transcript of the receptor protein gene normally found in the cell. This antisense RNA transcript of the inserted DNA can then base-pair with the normal mRNA transcript found in the cell and thereby prevent the mRNA from being translated. It is of course necessary to select sequences of the 5' flanking region that are downstream from the transcriptional start sites for the receptor protein gene to ensure that the antisense RNA contains complementary sequences present on the mRNA. Antisense RNA can be generated *in vitro* also, and then inserted into cells. Oligonucleotides can be synthesized on an automated synthesizer (e.g., Model 8700 automated synthesizer of Milligen-Biosearch, Burlington, MA or ABI Model 380B). In addition, antisense deoxyoligonucleotides have been shown to be effective in inhibiting gene transcription and viral replication (see e.g., Zamecnik et al., 1978 Proc. Natl. Acad. Sci. USA 75, 280-284; Zamecnik et al., 1986 Proc. Natl. Acad. Sci., 83, 4143-4146; Wickstrom et al., 1988 Proc. Natl. Acad. Sci. USA 85, 1028-1032; Crooke, 1993 FASEB J. 7, 533-539. Furthermore, recent work has shown that improved inhibition of expression of a gene by antisense oligonucleotides is possible if the antisense oligonucleotides contain modified nucleotides (see,

e.g., Offensperger et. al., 1993 EMBO J. 12, 1257-1262 (*in vivo* inhibition of duck hepatitis B viral replication and gene expression by antisense phosphorothioate oligodeoxynucleotides); Rosenberg et al., PCT WO 93/01286 (synthesis of sulfurthioate oligonucleotides); Agrawal et al., 1988 Proc. Natl. Acad. Sci. USA 85, 7079-7083 (synthesis of antisense oligonucleoside phosphoramidates and phosphorothioates to inhibit replication of human immunodeficiency virus-1); Sarin et al., 1989 Proc. Natl. Acad. Sci. USA 85, 7448-7794 (synthesis of antisense methylphosphonate oligonucleotides); Shaw et al., 1991 Nucleic Acids Res 19, 747-750 (synthesis of 3' exonuclease-resistant oligonucleotides containing 3' terminal phosphoroamidate modifications); incorporated herein by reference).

The sequences of the 5' flanking region of receptor protein gene can also be used in triple helix (triplex) gene therapy. Oligonucleotides complementary to gene promoter sequences on one of the strands of the DNA have been shown to bind promoter and regulatory sequences to form local triple nucleic acid helices which block transcription of the gene (see, e.g., 1989 Maher et al., Science 245, 725-730; Orson et al., 1991 Nucl. Acids Res. 19, 3435-3441; Postal et al., 1991 Proc. Natl. Acad. Sci. USA 88, 8227-8231; Cooney et al., 1988 Science 241, 456-459; Young et al., 1991 Proc. Natl. Acad. Sci. USA 88, 10023-10026; Duval-Valentin et al., 1992 Proc. Natl. Acad. Sci. USA 89, 504-508; 1992 Blume et al., Nucl. Acids Res. 20, 1777-1784; 1992 Grigoriev et al., J. Biol. Chem. 267, 3389-3395.

Recently, both theoretical calculations and empirical findings have been reported which provide guidance for the design of oligonucleotides for use in oligonucleotide-directed triple helix formation to inhibit gene expression. For example, oligonucleotides should generally be greater than 14 nucleotides in length to ensure target sequence

specificity (see, e.g., Maher et al., (1989); Grigoriev et al., (1992)). Also, many cells avidly take up oligonucleotides that are less than 50 nucleotides in length (see e.g., Orson et al., (1991); Holt et al., 1988 Mol. Cell. Biol. 8, 963-973; Wickstrom et al., 1988 Proc. Natl. Acad. Sci. USA 85, 1028-1032). To reduce susceptibility to intracellular degradation, for example by 3′ exonucleases, a free amine can be introduced to a 3′ terminal hydroxyl group of oligonucleotides without loss of sequence binding specificity (Orson et al., 1991). Furthermore, more stable triplexes are formed if any cytosines that may be present in the oligonucleotide are methylated, and also if an intercalating agent, such as an acridine derivative, is covalently attached to a 5′ terminal phosphate (e.g., via a pentamethylene bridge); again without loss of sequence specificity (Maher et al., (1989); Grigoriev et al., (1992).

Methods to produce or synthesize oligonucleotides are well known in the art. Such methods can range from standard enzymatic digestion followed by nucleotide fragment isolation (see e.g., Sambrook et al., Chapters 5, 6) to purely synthetic methods, for example, by the cyanoethyl phosphoramidite method using a Milligen or Beckman System 1Plus DNA synthesizer (see also, Ikuta et al., in Ann. Rev. Biochem. 1984 53, 323-356 (phosphotriester and phosphite-triester methods); Narang et al., in Methods Enzymol., 65, 610-620 (1980) (phosphotriester method). Accordingly, DNA sequences of the 5′ flanking region of the receptor protein gene described herein can be used to design and construct oligonucleotides including a DNA sequence consisting essentially of at least 15 consecutive nucleotides, with or without base modifications or intercalating agent derivatives, for use in forming triple helices specifically within the 5′ flanking region of a receptor protein gene in order to inhibit expression of the gene.

In some cases it may be advantageous to insert enhancers or multiple copies of the regulatory sequences into an expression system to facilitate screening of methods and reagents for manipulation of expression.

Preparation of Receptor Protein Fragments

Compounds which are effective for blocking binding of the receptor can also consist of fragments of the receptor proteins, expressed recombinantly and cleaved by enzymatic digest or expressed from a sequence encoding a peptide of less than the full length receptor protein. These will typically be soluble proteins, i.e., not including the transmembrane and cytoplasmic regions, although smaller portions determined in the assays described above to inhibit or compete for binding to the receptor proteins can also be utilized. It is a routine matter to make appropriate receptor protein fragments, test for binding, and then utilize. The preferred fragments are of human origin, in order to minimize potential immunological response. The peptides can be as short as five to eight amino acids in length and are easily prepared by standard techniques. They can also be modified to increase *in vivo* half-life, by chemical modification of the amino acids or by attachment to a carrier molecule or inert substrate. Based on studies with other peptide fragments blocking receptor binding, the IC_{50}, the dose of peptide required to inhibit binding by 50%, ranges from about 50 μM to about 300 μM, depending on the peptides. These ranges are well within the effective concentrations for the *in vivo* administration of peptides, based on comparison with the RGD-containing peptides, described, for example, in U.S. Patent No. 4,792,525 to Ruoslaghti, et al., used *in vivo* to alter cell attachment and phagocytosis. The peptides can also be conjugated to a carrier protein such as keyhole limpet hemocyanin by its N-terminal cysteine by standard procedures such as

the commercial Inject kit from Pierce Chemicals or expressed as a fusion protein, which may have increased efficacy. As noted above, the peptides can be prepared by proteolytic cleavage of the receptor proteins, or, preferably, by synthetic means. These methods are known to those skilled in the art. An example is the solid phase synthesis described by J. Merrifield, 1964 J. Am. Chem. Soc. 85, 2149, used in U.S. Patent No. 4,792,525, and described in U.S. Patent No. 4,244,946, wherein a protected alpha-amino acid is coupled to a suitable resin, to initiate synthesis of a peptide starting from the C-terminus of the peptide. Other methods of synthesis are described in U.S. Patent No. 4,305,872 and 4,316,891. These methods can be used to synthesize peptides having identical sequence to the receptor proteins described herein, or substitutions or additions of amino acids, which can be screened for activity as described above.

The peptide can also be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

Peptides containing cyclopropyl amino acids, or amino acids derivatized in a similar fashion, can also be used. These peptides retain their original activity but have increased half-lives *in vivo*. Methods known for modifying amino acids, and their use, are known to those skilled in the art, for

example, as described in U.S. Patent No. 4,629,784 to Stammer.

The peptides are generally active when administered parenterally in amounts above about 1 μg/kg of body weight. Based on extrapolation from other proteins, for treatment of most inflammatory disorders, the dosage range will be between 0.1 to 70 mg/kg of body weight. This dosage will be dependent, in part, on whether one or more peptides are administered.

Pharmaceutical Compositions

Compounds which alter receptor protein binding are preferably administered in a pharmaceutically acceptable vehicle. Suitable pharmaceutical vehicles are known to those skilled in the art. For parenteral administration, the compound will usually be dissolved or suspended in sterile water or saline. For enteral administration, the compound will be incorporated into an inert carrier in tablet, liquid, or capsular form. Suitable carriers may be starches or sugars and include lubricants, flavorings, binders, and other materials of the same nature. The compounds can also be administered locally by topical application of a solution, cream, gel, or polymeric material (for example, a Pluronic™, BASF).

Alternatively, the compound may be administered in liposomes or microspheres (or microparticles). Methods for preparing liposomes and microspheres for administration to a patient are known to those skilled in the art. U.S. Patent No. 4,789,734 describe methods for encapsulating biological materials in liposomes. Essentially, the material is dissolved in an aqueous solution, the appropriate phospholipids and lipids added, along with surfactants if required, and the material dialyzed or sonicated, as necessary. A review of known methods is by G. Gregoriadis, Chapter 14. "Liposomes", <u>Drug Carriers in Biology and Medicine</u> pp. 287-341 (Academic Press, 1979). Microspheres formed of polymers or proteins are well

known to those skilled in the art, and can be tailored for passage through the gastrointestinal tract directly into the bloodstream. Alternatively, the compound can be incorporated and the microspheres, or composite of microspheres, implanted for slow release over a period of time, ranging from days to months. See, for example, U.S. Patent No. 4,906,474, 4,925,673, and 3,625,214.

Removal of LDL from patients or patient samples

The SR-BI receptor proteins can be used to remove LDL from patient blood, by immobilizing the receptor on a suitable substrate, such as the cellulose membrane of a dialysis unit, using conventional coupling, for example, using carboimide. The patient's blood is then dialyzed through the unit.

Modifications and variations of the methods and materials described herein will be obvious to those skilled in the art and are intended to be encompassed by the following claims. The teachings of the references cited herein are specifically incorporated herein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:
(i) APPLICANT: Massachusetts Institute of Technology
(ii) TITLE OF INVENTION: Class BI and CI Scavenger Receptors
(iii) NUMBER OF SEQUENCES: 8
(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Patrea L. Pabst
(B) STREET: 2800 One Atlantic Center
1201 West Peachtree Street
(C) CITY: Atlanta
(D) STATE: Georgia
(E) COUNTRY: USA
(F) ZIP: 30309-3450
(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Pabst, Patrea L.
(B) REGISTRATION NUMBER: 31,284
(C) REFERENCE/DOCKET NUMBER: MIT6620
(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (404) 873-8794
(B) TELEFAX: (404) 873-8795
(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Ashkenas, et al.
(C) JOURNAL: J. Lipid Res.
(D) VOLUME: 34
(F) PAGES: 983-1000
(G) DATE: 1993
(K) RELEVANT RESIDUES IN SEQ ID NO:1: FROM 1 TO 20
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Ashkenas, et al.
(C) JOURNAL: J. Lipid Res.
(D) VOLUME: 34
(F) PAGES: 983-1000
(G) DATE: 1993
(K) RELEVANT RESIDUES IN SEQ ID NO:1: FROM 1 TO 18
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(2) INFORMATION FOR SEQ ID NO:3:
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(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
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(B) LOCATION: 156..1683
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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CCTGAGCCCC GCGAGCCCGG GCCGCACACG CGGACATGGG CGGCAGCGCC AGGGCGCGCT    180
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    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Pro Ser Leu Ile Lys Gln Gln Val Leu Lys Asn Val Arg Ile Asp Pro
        35                  40                  45

Ser Ser Leu Ser Phe Ala Met Trp Lys Glu Ile Pro Val Pro Phe Tyr
    50                  55                  60

Leu Ser Val Tyr Phe Phe Glu Val Val Asn Pro Ser Glu Ile Leu Lys
65                  70                  75                  80

Gly Glu Lys Pro Val Val Arg Glu Arg Gly Pro Tyr Val Tyr Arg Glu
                85                  90                  95

Phe Arg His Lys Ala Asn Ile Thr Phe Asn Asp Asn Asp Thr Val Ser
            100                 105                 110

Phe Val Glu His Arg Ser Leu His Phe Gln Pro Asp Arg Ser His Gly
        115                 120                 125
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Ser Glu Ser Asp Tyr Ile Ile Leu Pro Asn Ile Leu Val Leu Gly Gly
130 135 140

Ala Val Met Met Glu Ser Lys Ser Ala Gly Leu Lys Leu Met Met Thr
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Thr Ser Gly Gln Met Trp Ala Pro Phe Met Thr Pro Gln Ser Ser Leu
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Glu Phe Phe Ser Pro Glu Ala Cys Arg Ser Met Lys Leu Thr Tyr His
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Cys Pro Cys Leu Glu Ser Gly Ile Gln Asn Val Ser Thr Cys Arg Phe
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Gly Ala Pro Leu Phe Leu Ser His Pro His Phe Tyr Asn Ala Asp Pro
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Ser Val Lys Leu Gln Ile Ser Leu Tyr Ile Lys Ala Val Lys Gly Ile
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Glu Gln Ser Gly Ala Met Gly Gly Glu Pro Leu Asn Thr Phe Tyr Thr
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Gln Leu Val Leu Met Pro Gln Val Leu Gln Tyr Val Gln Tyr Val Leu
435 440 445

Leu Gly Leu Gly Gly Leu Leu Leu Leu Val Pro Val Ile Tyr Gln Leu
450 455 460

Arg Ser Gln Glu Lys Cys Phe Leu Phe Trp Ser Gly Ser Lys Lys Gly
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Ser Gln Asp Lys Glu Ala Ile Gln Ala Tyr Ser Glu Ser Leu Met Ser
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Pro Ala Ala Lys Gly Thr Val Leu Gln Glu Ala Lys Leu

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(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(ix) FEATURE:
(A) NAME/KEY: misc. feature
(B) LOCATION: 40..1926
(D) OTHER INFORMATION: /Function = "Nucleotides 40 through 1926 encode the amino acid sequence for the Drosophila Melanogaster Scavenger Receptor Class CI."
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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TTGGATAATG GAAGTATAAA TTATCGACAG AGAAATATAG TGAGATTCAG ATGCAATCGC 180

GGCTACACTT TGCAGGGAAC AGTAATGCAA ACTTGCGATC GAGATGGTCG CCTTCGAGGC 240

GAAAAACCAT TCTGTGCCAG TAGGGGATGT GCGAGGCCCG AGGATCCGGA GAACGGACAC 300

GTCGAAAATC TTTCCCTAAG GGCGGATGTC GTGTGCCACG ATGGCTATGT CTTGGTCGGT 360

GGTCGCACTG CCTACTGCGA TGGAGAAAGA TGGAGCACCC AGCTGGGATC GTGTCGAAGG 420

AGCAACCACA CAAGAGATCA TTCTTGCGAT TTCGAGAGCG AGGATCAGTG CGGTTGGGAG 480

GCGGAGACAA CCTTCCGACG ACCCTGGAAG CGAGTCAGCA CGGTATCCGA TATTCACTCC 540

CTAAGAACGG GACCCCGCCA CGATCACACG TTTAAAAACG AATCCGGTGG TCATTACATG 600

CGCATGGAAA CCCAAATGGG GGCTTATGGA AGCTACCATC TGCTATCGCC GATCTATCCC 660

AGATCCCTCA CCCTGAAGAC CGCCTGCTGC TTTCGATTCC ACTACTTCAT GTTTGGCGCT 720

GGTGTGGATA ATCTGGTGGT GTCCGTTAAA CCCGTTTCGA TGCCAATGGC AACCATGTGG 780

AATAGGTTCA GAGCCAATTG CAGCAAATTT GAGATATCTG GTCAGCAGGG AACCCAGTGG 840

CTAGAGCACA CGATCAGCAT TGACGAGATG CAAGAGGACT TCCAGGTGAT ATTCACGGCA 900

ACGGATGCAA GATCCCAATT CGGAGATATT GCCATCGATG ATGTAAAGCT AATGACAGGC 960

AGTGAGTGTG GCACAAACGG ATTTAGCACC ACCACAGAAC CAACGGCTCC GACAGGCAGC 1020

AACGAGCAAC CACTGGTCTA CGATATGATA AGTTGTTCAG GTCGATGCGG AACATCAATG 1080

TCGGCCTCCA ATATAACCAA CAATGGTATA GTCATGGGAT GTGGATGTAA TGACGAGTGC 1140

CTTTCGGATG AGACTTGTTG CCTAAACTAT TTGGAGGAGT GCACAAAGGA GCTGCTCACC 1200

ACGACCGAGG ATGATATTAG TTCCCTGCCC CCAACGGTCA CATCAACAAG CACAAGCACT 1260

ACGAGGAAGT CAACAACAAC AACAACCACA AGCACGACTA CTACAAGTAC AACAACAACT 1320

AAAAGGCCAA CCACAACCAC AACAACAACA AAGGCCACAA CTACAAAGCG AACAACAACC 1380

ACTAAAAAAC CGACAACAAC TTCAACAACG CCGAAGCCAA CAACAACGAC TTCAACCACA 1440

CCAAAGTCTA CAACTTCTAC AACGTCTACA ACTTCAACAA CACCAACGAC AACAACTACA 1500

ATAAATGTGT TTACAACAAA GAAAACAACA ATAATGATCC CTACTTCCAG TACCGAAAAG 1560

ACTACAGGCA TCATCACCAC CATGAAGACA CGCAAGCGCA TCACTTGGAA CGTTGATCCT 1620

CAGGACATCG AGGGTCACAT GGACACGAGC GGAAGTACCC CCAATCCAGC TTTAGTAGTA 1680

CTTTACCTGC TACTCGGCAT TGTTCTGGTG GTAGTTCTGG CCAACGTCGT TAATCGCTGG 1740

ATAATACCAA TCACTGGATC AAAGACCAGC AGCGAAAAGG CTGTGAGATT CAAGAAGGCA 1800

TTCGATAGTC TGAAGAAGCA ACGGAAAAGA AACAGCATGG ATGATCAGCC GTTATGCGAC 1860

TCCGATAACG ACGATGTAGA GTATTTCGAA GAAATGGGCG TGGACATACG ACATAGGACC 1920

GATCTATGAG GGTAATCCCC AGTGATACCA AAACAAACGC TTAGGCCTGT GCCTATTGTA 1980

TAGGATGTTT CTAAATGTGT ATGCAAGAAT CGAATAAAAG AAAATATGCA AC 2032

(2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 629 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(v) FRAGMENT TYPE: internal
(ix) FEATURE:
(A) NAME/KEY: misc. feature
(B) LOCATION: 1..629
(D) OTHER INFORMATION: /Function = "Amino acid sequence for the Drosophila Melanogaster Scavenger Receptor Class CI."
(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 30..353
(D) OTHER INFORMATION: /note= "Positions 30-32, 90-92, 129-131, 180-182, 253-255 and 351-353 represent potential N-glycosylation sites."
(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1..20
(D) OTHER INFORMATION: /note= "Amino acids 1-20 represent a putative signal sequence."
(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 21..74
(D) OTHER INFORMATION: /note= "Amino acids 21-74 represent complement control protein domain number 1."
(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 75..127
(D) OTHER INFORMATION: /note= "Amino acids 75-127 represent complement control protein domain number 2."
(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 128..312
(D) OTHER INFORMATION: /note= "Amino acids 128-312 represent an MAM domain."
(ix) FEATURE:
(A) NAME/KEY: Disulfide-bond
(B) LOCATION: 22..381
(D) OTHER INFORMATION: /note= "The cysteines at positions 22, 45, 59, 72, 77, 99, 113, 125, 136, 144, 216, 217, 254, 310, 339, 343, 361, 363, 367, 373, 374 and 381 represent potential disulfide linkages."
(ix) FEATURE:
(A) NAME/KEY: Modified-site

(B) LOCATION: 338..381
(D) OTHER INFORMATION: /note= "Amino acids 338-381 represent a somatomedin B domain."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 387..514
(D) OTHER INFORMATION: /note= "Amino acids 387-514 represent a mucin-like potential O-linked glycosylation region."

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 544..564
(D) OTHER INFORMATION: /note= "Amino acids 544-565 represent a putative TM domain."

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 565..629
(D) OTHER INFORMATION: /note= "Amino acids 565-629 represent a putative cytoplasmic domain."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 576..602
(D) OTHER INFORMATION: /note= "Amino acids 576-579 and 599-602 represent casein kinase II sites."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 578..592
(D) OTHER INFORMATION: /note= "Amino acids 578-580 and 590-592 represent protein kinase C sites."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 596..599
(D) OTHER INFORMATION: /note= "Amino acids 596-599 represent a cAMP protein kinase site."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Glu Phe Phe Trp Thr Leu Ala Val Ile Val Ile Tyr Cys Ile Gly
1               5                   10                  15

His Ile His Gly Arg Cys Glu Arg Ser Ile Asp Leu Asp Asn Gly Ser
            20                  25                  30

Ile Asn Tyr Arg Gln Arg Asn Ile Val Arg Phe Arg Cys Asn Arg Gly
        35                  40                  45

Tyr Thr Leu Gln Gly Thr Val Met Gln Thr Cys Asp Arg Asp Gly Arg
    50                  55                  60

Leu Arg Gly Glu Lys Pro Phe Cys Ala Ser Arg Gly Cys Ala Arg Pro
65                  70                  75                  80

Glu Asp Pro Glu Asn Gly His Val Glu Asn Leu Ser Leu Arg Ala Asp
                85                  90                  95

Val Val Cys His Asp Gly Tyr Val Leu Val Gly Gly Arg Thr Ala Tyr
            100                 105                 110

Cys Asp Gly Glu Arg Trp Ser Thr Gln Leu Gly Ser Cys Arg Arg Ser
        115                 120                 125

Asn His Thr Arg Asp His Ser Cys Asp Phe Glu Ser Glu Asp Gln Cys
    130                 135                 140

Gly Trp Glu Ala Glu Thr Thr Phe Arg Arg Pro Trp Lys Arg Val Ser
145                 150                 155                 160

Thr Val Ser Asp Ile His Ser Leu Arg Thr Gly Pro Arg His Asp His
```

165 170 175

Thr Phe Lys Asn Glu Ser Gly Gly His Tyr Met Arg Met Glu Thr Gln
180 185 190

Met Gly Ala Tyr Gly Ser Tyr His Leu Leu Ser Pro Ile Tyr Pro Arg
195 200 205

Ser Leu Thr Leu Lys Thr Ala Cys Cys Phe Arg Phe His Tyr Phe Met
210 215 220

Phe Gly Ala Gly Val Asp Asn Leu Val Val Ser Val Lys Pro Val Ser
225 230 235 240

Met Pro Met Ala Thr Met Trp Asn Arg Phe Arg Ala Asn Cys Ser Lys
245 250 255

Phe Glu Ile Ser Gly Gln Gln Gly Thr Gln Trp Leu Glu His Thr Ile
260 265 270

Ser Ile Asp Glu Met Gln Glu Asp Phe Gln Val Ile Phe Thr Ala Thr
275 280 285

Asp Ala Arg Ser Gln Phe Gly Asp Ile Ala Ile Asp Asp Val Lys Leu
290 295 300

Met Thr Gly Ser Glu Cys Gly Thr Asn Gly Phe Ser Thr Thr Thr Glu
305 310 315 320

Pro Thr Ala Pro Thr Gly Ser Asn Glu Gln Pro Leu Val Tyr Asp Met
325 330 335

Ile Ser Cys Ser Gly Arg Cys Gly Thr Ser Met Ser Ala Ser Asn Ile
340 345 350

Thr Asn Asn Gly Ile Val Met Gly Cys Gly Cys Asn Asp Glu Cys Leu
355 360 365

Ser Asp Glu Thr Cys Cys Leu Asn Tyr Leu Glu Glu Cys Thr Lys Glu
370 375 380

Leu Leu Thr Thr Thr Glu Asp Asp Ile Ser Ser Leu Pro Pro Thr Val
385 390 395 400

Thr Ser Thr Ser Thr Ser Thr Thr Arg Lys Ser Thr Thr Thr Thr Thr
405 410 415

Thr Ser Thr Thr Thr Thr Ser Thr Thr Thr Thr Lys Arg Pro Thr Thr
420 425 430

Thr Thr Thr Thr Thr Lys Ala Thr Thr Thr Lys Arg Thr Thr Thr Thr
435 440 445

Lys Lys Pro Thr Thr Thr Ser Thr Thr Pro Lys Pro Thr Thr Thr Thr
450 455 460

Ser Thr Thr Pro Lys Ser Thr Thr Ser Thr Thr Ser Thr Thr Ser Thr
465 470 475 480

Thr Pro Thr Thr Thr Thr Thr Ile Asn Val Phe Thr Thr Lys Lys Thr
485 490 495

Thr Ile Met Ile Pro Thr Ser Ser Thr Glu Lys Thr Thr Gly Ile Ile
500 505 510

Thr Thr Met Lys Thr Arg Lys Arg Ile Thr Trp Asn Val Asp Pro Gln
515 520 525

Asp Ile Glu Gly His Met Asp Thr Ser Gly Ser Thr Pro Asn Pro Ala
530 535 540

Leu Val Val Leu Tyr Leu Leu Leu Gly Ile Val Leu Val Val Val Leu
545 550 555 560

Ala Asn Val Val Asn Arg Trp Ile Ile Pro Ile Thr Gly Ser Lys Thr
565 570 575

Ser Ser Glu Lys Ala Val Arg Phe Lys Lys Ala Phe Asp Ser Leu Lys
580 585 590

Lys Gln Arg Lys Arg Asn Ser Met Asp Asp Gln Pro Leu Cys Asp Ser
595 600 605

Asp Asn Asp Asp Val Glu Tyr Phe Glu Glu Met Gly Val Asp Ile Arg
610 615 620

His Arg Thr Asp Leu
625

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1785 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 51..1577

(D) OTHER INFORMATION: /Function = "Nucleotides 51 through 1577 encode the amino acid sequence for the murine Scavenger Receptor Class BI."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCGTCTCCTT CAGGTCCTGA GCCCCGAGAG CCCCTTCCGC GCACGCGGAC ATG GGC 56
Met Gly
1

GGC AGC TCC AGG GCG CGC TGG GTG GCC TTG GGG TTG GGC GCC CTG GGG 104
Gly Ser Ser Arg Ala Arg Trp Val Ala Leu Gly Leu Gly Ala Leu Gly
5 10 15

CTG CTG TTT GCT GCG CTC GGC GTT GTC ATG ATC CTC ATG GTG CCC TCC 152
Leu Leu Phe Ala Ala Leu Gly Val Val Met Ile Leu Met Val Pro Ser
20 25 30

CTC ATC AAG CAG CAG GTG CTC AAG AAT GTC CGC ATA GAC CCG AGC AGC 200
Leu Ile Lys Gln Gln Val Leu Lys Asn Val Arg Ile Asp Pro Ser Ser
35 40 45 50

CTG TCC TTC GGG ATG TGG AAG GAG ATC CCC GTC CCT TTC TAC TTG TCT 248
Leu Ser Phe Gly Met Trp Lys Glu Ile Pro Val Pro Phe Tyr Leu Ser
55 60 65

GTC TAC TTC TTC GAA GTG GTC AAC CCA AAC GAG GTC CTC AAC GGC CAG 296
Val Tyr Phe Phe Glu Val Val Asn Pro Asn Glu Val Leu Asn Gly Gln
70 75 80

AAG CCA GTA GTC CGG GAG CGT GGA CCC TAT GTC TAC AGG GAG TTC AGA 344
Lys Pro Val Val Arg Glu Arg Gly Pro Tyr Val Tyr Arg Glu Phe Arg
85 90 95

CAA AAG GTC AAC ATC ACC TTC AAT GAC AAC GAC ACC GTG TCC TTC GTG 392
Gln Lys Val Asn Ile Thr Phe Asn Asp Asn Asp Thr Val Ser Phe Val
100 105 110

GAG AAC CGC AGC CTC CAT TTC CAG CCT GAC AAG TCG CAT GGC TCA GAG 440
Glu Asn Arg Ser Leu His Phe Gln Pro Asp Lys Ser His Gly Ser Glu
115 120 125 130

AGT GAC TAC ATT GTA CTG CCT AAC ATC TTG GTC CTG GGG GGC TCG ATA 488
Ser Asp Tyr Ile Val Leu Pro Asn Ile Leu Val Leu Gly Gly Ser Ile
135 140 145

TTG ATG GAG AGC AAG CCT GTG AGC CTG AAG CTG ATG ATG ACC TTG GCG 536
Leu Met Glu Ser Lys Pro Val Ser Leu Lys Leu Met Met Thr Leu Ala
150 155 160

CTG GTC ACC ATG GGC CAG CGT GCT TTT ATG AAC CGC ACA GTT GGT GAG 584
Leu Val Thr Met Gly Gln Arg Ala Phe Met Asn Arg Thr Val Gly Glu
165 170 175

ATC CTG TGG GGC TAT GAC GAT CCC TTC GTG CAT TTT CTC AAC ACG TAC 632
Ile Leu Trp Gly Tyr Asp Asp Pro Phe Val His Phe Leu Asn Thr Tyr
180 185 190

CTC CCA GAC ATG CTT CCC ATA AAG GGC AAA TTT GGC CTG TTT GTT GGG 680
Leu Pro Asp Met Leu Pro Ile Lys Gly Lys Phe Gly Leu Phe Val Gly
195 200 205 210

ATG AAC AAC TCG AAT TCT GGG GTC TTC ACT GTC TTC ACG GGC GTC CAG 728
Met Asn Asn Ser Asn Ser Gly Val Phe Thr Val Phe Thr Gly Val Gln
215 220 225

AAT TTC AGC AGG ATC CAT CTG GTG GAC AAA TGG AAC GGA CTC AGC AAG 776
Asn Phe Ser Arg Ile His Leu Val Asp Lys Trp Asn Gly Leu Ser Lys
230 235 240

ATC GAT TAT TGG CAT TCA GAG CAG TGT AAC ATG ATC AAT GGG ACT TCC 824
Ile Asp Tyr Trp His Ser Glu Gln Cys Asn Met Ile Asn Gly Thr Ser
245 250 255

GGG CAG ATG TGG GCA CCC TTC ATG ACA CCC GAA TCC TCG CTG GAA TTC 872
Gly Gln Met Trp Ala Pro Phe Met Thr Pro Glu Ser Ser Leu Glu Phe
260 265 270

TTC AGC CCG GAG GCA TGC AGG TCC ATG AAG CTG ACC TAC AAC GAA TCA 920
Phe Ser Pro Glu Ala Cys Arg Ser Met Lys Leu Thr Tyr Asn Glu Ser
275 280 285 290

AGG GTG TTT GAA GGC ATT CCC ACG TAT CGC TTC ACG GCC CCC GAT ACT 968
Arg Val Phe Glu Gly Ile Pro Thr Tyr Arg Phe Thr Ala Pro Asp Thr
295 300 305

CTG TTT GCC AAC GGG TCC GTC TAC CCA CCC AAC GAA GGC TTC TGC CCA 1016
Leu Phe Ala Asn Gly Ser Val Tyr Pro Pro Asn Glu Gly Phe Cys Pro
310 315 320

TGC CGA GAG TCT GGC ATT CAG AAT GTC AGC ACC TGC AGG TTT GGT GCG 1064
Cys Arg Glu Ser Gly Ile Gln Asn Val Ser Thr Cys Arg Phe Gly Ala
325 330 335

CCT CTG TTT CTC TCC CAC CCC CAC TTT TAC AAC GCC GAC CCT GTG TTG 1112
Pro Leu Phe Leu Ser His Pro His Phe Tyr Asn Ala Asp Pro Val Leu
340 345 350

TCA GAA GCT GTT CTT GGT CTG AAC CCT AAC CCA AAG GAG CAT TCC TTG 1160
Ser Glu Ala Val Leu Gly Leu Asn Pro Asn Pro Lys Glu His Ser Leu
355 360 365 370

TTC CTA GAC ATC CAT CCG GTC ACT GGG ATC CCC ATG AAC TGT TCT GTG 1208
Phe Leu Asp Ile His Pro Val Thr Gly Ile Pro Met Asn Cys Ser Val
375 380 385

AAG ATG CAG CTG AGC CTC TAC ATC AAA TCT GTC AAG GGC ATC GGG CAA 1256
Lys Met Gln Leu Ser Leu Tyr Ile Lys Ser Val Lys Gly Ile Gly Gln

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                390                     395                     400

ACA GGG AAG ATC GAG CCA GTA GTT CTG CCG TTG CTG TGG TTC GAA CAG      1304
Thr Gly Lys Ile Glu Pro Val Val Leu Pro Leu Leu Trp Phe Glu Gln
        405                     410                     415

AGC GGA GCA ATG GGT GGC AAG CCC CTG AGC ACG TTC TAC ACG CAG CTG      1352
Ser Gly Ala Met Gly Gly Lys Pro Leu Ser Thr Phe Tyr Thr Gln Leu
    420                     425                     430

GTG CTG ATG CCC CAG GTT CTT CAC TAC GCG CAG TAT GTG CTG CTG GGG      1400
Val Leu Met Pro Gln Val Leu His Tyr Ala Gln Tyr Val Leu Leu Gly
435                     440                     445                     450

CTT GGA GGC CTC CTG TTG CTG GTG CCC ATC ATC TGC CAA CTG CGC AGC      1448
Leu Gly Gly Leu Leu Leu Leu Val Pro Ile Ile Cys Gln Leu Arg Ser
                455                     460                     465

CAG GAG AAA TGC TTT TTG TTT TGG AGT GGT AGT AAA AAG GGC TCC CAG      1496
Gln Glu Lys Cys Phe Leu Phe Trp Ser Gly Ser Lys Lys Gly Ser Gln
            470                     475                     480

GAT AAG GAG GCC ATT CAG GCC TAC TCT GAG TCC CTG ATG TCA CCA GCT      1544
Asp Lys Glu Ala Ile Gln Ala Tyr Ser Glu Ser Leu Met Ser Pro Ala
        485                     490                     495

GCC AAG GGC ACG GTG CTG CAA GAA GCC AAG CTA TAGGGTCCTG AAGACACTAT    1597
Ala Lys Gly Thr Val Leu Gln Glu Ala Lys Leu
    500                     505

AAGCCCCCCA AACCTGATAG CTTGGTCAGA CCAGCCACCC AGTCCCTACA CCCCGCTTCT    1657

TGAGGACTCT CTCAGCGGAC AGCCCACCAG TGCCATGGCC TGAGCCCCCA GATGTCACAC    1717

CTGTCCGCAC GCACGGCACA TGGATGCCCA CGCATGTGCA AAAACAACTC AGGGACCAGG    1777

GACAGACC                                                             1785
```

(2) INFORMATION FOR SEQ ID NO:8:
- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 509 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (ix) FEATURE:
  - (A) NAME/KEY: misc. feature
  - (B) LOCATION: 1..509
  - (D) OTHER INFORMATION: /Function = "Amino acid sequence for the murine Scavenger Receptor Class BI."
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Gly Gly Ser Ser Arg Ala Arg Trp Val Ala Leu Gly Leu Gly Ala
  1               5                  10                  15

Leu Gly Leu Leu Phe Ala Ala Leu Gly Val Val Met Ile Leu Met Val
             20                  25                  30

Pro Ser Leu Ile Lys Gln Gln Val Leu Lys Asn Val Arg Ile Asp Pro
         35                  40                  45

Ser Ser Leu Ser Phe Gly Met Trp Lys Glu Ile Pro Val Pro Phe Tyr
     50                  55                  60

Leu Ser Val Tyr Phe Phe Glu Val Val Asn Pro Asn Glu Val Leu Asn
 65                  70                  75                  80

Gly Gln Lys Pro Val Val Arg Glu Arg Gly Pro Tyr Val Tyr Arg Glu
                 85                  90                  95

Phe Arg Gln Lys Val Asn Ile Thr Phe Asn Asp Asn Asp Thr Val Ser
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100 105 110

Phe Val Glu Asn Arg Ser Leu His Phe Gln Pro Asp Lys Ser His Gly
115 120 125

Ser Glu Ser Asp Tyr Ile Val Leu Pro Asn Ile Leu Val Leu Gly Gly
130 135 140

Ser Ile Leu Met Glu Ser Lys Pro Val Ser Leu Lys Leu Met Met Thr
145 150 155 160

Leu Ala Leu Val Thr Met Gly Gln Arg Ala Phe Met Asn Arg Thr Val
165 170 175

Gly Glu Ile Leu Trp Gly Tyr Asp Asp Pro Phe Val His Phe Leu Asn
180 185 190

Thr Tyr Leu Pro Asp Met Leu Pro Ile Lys Gly Lys Phe Gly Leu Phe
195 200 205

Val Gly Met Asn Asn Ser Asn Ser Gly Val Phe Thr Val Phe Thr Gly
210 215 220

Val Gln Asn Phe Ser Arg Ile His Leu Val Asp Lys Trp Asn Gly Leu
225 230 235 240

Ser Lys Ile Asp Tyr Trp His Ser Glu Gln Cys Asn Met Ile Asn Gly
245 250 255

Thr Ser Gly Gln Met Trp Ala Pro Phe Met Thr Pro Glu Ser Ser Leu
260 265 270

Glu Phe Phe Ser Pro Glu Ala Cys Arg Ser Met Lys Leu Thr Tyr Asn
275 280 285

Glu Ser Arg Val Phe Glu Gly Ile Pro Thr Tyr Arg Phe Thr Ala Pro
290 295 300

Asp Thr Leu Phe Ala Asn Gly Ser Val Tyr Pro Pro Asn Glu Gly Phe
305 310 315 320

Cys Pro Cys Arg Glu Ser Gly Ile Gln Asn Val Ser Thr Cys Arg Phe
325 330 335

Gly Ala Pro Leu Phe Leu Ser His Pro His Phe Tyr Asn Ala Asp Pro
340 345 350

Val Leu Ser Glu Ala Val Leu Gly Leu Asn Pro Asn Pro Lys Glu His
355 360 365

Ser Leu Phe Leu Asp Ile His Pro Val Thr Gly Ile Pro Met Asn Cys
370 375 380

Ser Val Lys Met Gln Leu Ser Leu Tyr Ile Lys Ser Val Lys Gly Ile
385 390 395 400

Gly Gln Thr Gly Lys Ile Glu Pro Val Val Leu Pro Leu Leu Trp Phe
405 410 415

Glu Gln Ser Gly Ala Met Gly Gly Lys Pro Leu Ser Thr Phe Tyr Thr
420 425 430

Gln Leu Val Leu Met Pro Gln Val Leu His Tyr Ala Gln Tyr Val Leu
435 440 445

Leu Gly Leu Gly Gly Leu Leu Leu Leu Val Pro Ile Ile Cys Gln Leu
450 455 460

Arg Ser Gln Glu Lys Cys Phe Leu Phe Trp Ser Gly Ser Lys Lys Gly
465 470 475 480

```
Ser Gln Asp Lys Glu Ala Ile Gln Ala Tyr Ser Glu Ser Leu Met Ser
                485                 490                 495

Pro Ala Ala Lys Gly Thr Val Leu Gln Glu Ala Lys Leu
            500                 505
```

We claim:

1. An isolated scavenger receptor protein type BI which selectively binds to low density lipoprotein and to modified lipoprotein having the characteristics of acetylated low density lipoprotein.

2. The protein of claim 1 expressed in cells selected from the group consisting of adipocytes, lung and liver.

3. The protein of claim 1 encoded by a sequence hybridizing under stringent conditions to Sequence ID No. 3.

4. The protein of claim 3 wherein the sequence is Sequence ID No. 3 or a degenerate variant thereof.

5. The protein of claim 1 having an amino acid sequence consisting essentially of the sequence shown in Sequence ID No. 4.

6. The protein of claim 1 immobilized to an inert substrate in a form useful for binding of low density lipoprotein.

7. The protein of claim 1 of human origin.

8. The protein of claim 1 expressed on the surface of a cell genetically engineered to express the protein.

9. An antibody to scavenger receptor protein which selectively binds to low density lipoprotein and to modified lipoprotein having the characteristics of acetylated low density lipoprotein.

10. The antibody of claim 9 further comprising a detectable label.

11. An isolated nucleic acid sequence comprising at least fourteen nucleotides encoding at least in part or regulating the expression of a scavenger receptor protein type BI which selectively binds to low density lipoprotein and to modified lipoprotein having the characteristics of acetylated low density lipoprotein.

12. The sequence of claim 11 expressed in cells selected from the group consisting of adipocytes, lung and liver.

13. The sequence of claim 11 hybridizing under stringent conditions to Sequence ID No. 3.

14. The sequence of claim 13 wherein the sequence is Sequence ID No. 3 or a degenerate variant thereof.

15. The sequence of claim 11 encoding an amino acid sequence consisting essentially of the sequence shown in Sequence ID No. 4.

16. The sequence of claim 11 regulating expression of genomic DNA encoding the scavenger receptor protein.

17. The sequence of claim 11 encoding the scavenger receptor protein.

18. The sequence of claim 11 which is genomic DNA.

19. The sequence of claim 11 which encodes the human scavenger receptor.

20. The sequence of claim 11 labeled with a detectable label.

21. The sequence of claim 11 encoding the scavenger receptor protein further comprising an expression vector.

22. The sequence of claim 21 further comprising a host cell suitable for expression of the scavenger receptor.

23. An isolated scavenger receptor protein type CI which selectively binds to modified lipoprotein having the characteristics of acetylated low density lipoprotein and having the structure of the scavenger receptor protein defined by Sequence ID No. 6.

24. The protein of claim 23 expressed in *Drosophila melanogaster* cells.

25. The protein of claim 23 encoded by a sequence hybridizing under stringent conditions to Sequence ID No. 5.

26. The protein of claim 25 wherein the sequence is Sequence ID No. 5 or a degenerate variant thereof.

27. The protein of claim 23 having an amino acid sequence consisting essentially of the sequence shown in Sequence ID No. 6.

28. The protein of claim 23 of human origin.

29. The protein of claim 23 expressed on the surface of a cell genetically engineered to express the protein.

30. An antibody to scavenger receptor protein which selectively binds to modified lipoprotein having the characteristics of acetylated low density lipoprotein and having the structure of the scavenger receptor protein defined by Sequence ID No. 6.

31. The antibody of claim 30 further comprising a detectable label.

32. An isolated nucleic acid sequence comprising at least fourteen nucleotides encoding at least in part or regulating the expression of a scavenger receptor protein type CI which selectively binds to modified lipoprotein having the characteristics of acetylated low density lipoprotein and having the structure of the scavenger receptor protein defined by Sequence ID No. 6.

33. The sequence of claim 32 expressed in cells of *Drosophila melanogaster.*

34. The sequence of claim 32 hybridizing under stringent conditions to Sequence ID No. 5.

35. The sequence of claim 34 wherein the sequence is Sequence ID No. 5 or a degenerate variant thereof.

36. The sequence of claim 33 encoding an amino acid sequence consisting essentially of the sequence shown in Sequence ID No. 6.

37. The sequence of claim 33 regulating expression of genomic DNA encoding the scavenger receptor protein.

38. The sequence of claim 33 encoding the scavenger receptor protein.

39. The sequence of claim 33 which is genomic DNA.

40. The sequence of claim 33 which encodes the human scavenger receptor.

41. The sequence of claim 33 labeled with a detectable label.

42. The sequence of claim 33 encoding the scavenger receptor protein further comprising an expression vector.

43. The sequence of claim 42 further comprising a host cell suitable for expression of the scavenger receptor.

44. A method for screening for a compound which alters the binding of a scavenger receptor protein selected from the group consisting of scavenger receptor protein type BI which selectively binds to low density lipoprotein and to modified lipoprotein having the characteristics of acetylated low density lipoprotein and scavenger receptor protein type CI which selectively binds to modified lipoprotein having the characteristics of acetylated low density lipoprotein and having the structure of the scavenger receptor protein defined by Sequence ID No. 6 comprising

providing an assay for binding of low density lipoprotein or modified low density lipoprotein to the scavenger receptor protein,

adding the compound to be tested to the assay, and

determining if the amount of modified low density lipoprotein or low density lipoprotein which is bound to the scavenger receptor protein is altered as compared to binding in the absence of the compound to be tested.

45. The assay of claim 44 wherein the assay includes a cell expressing the scavenger receptor protein and the compound is a nucleic acid sequence which alters expression of the scavenger receptor protein.

46. The assay of claim 44 wherein the compound is selected from a library of naturally occurring or synthetic compounds which are randomly tested for alteration of binding.

47. The assay of claim 44 wherein the compound competitively inhibits binding to the scavenger receptor protein.

48. A method for removing low density lipoprotein from patient blood comprising reacting the blood with immobilized scavenger receptor protein type BI which selectively binds to low density lipoprotein and to modified lipoprotein having the characteristics of acetylated low density lipoprotein under conditions wherein the low density lipoprotein is bound to the scavenger receptor.

49. A method for inhibiting uptake of lipoprotein or lipids by adipocytes comprising selectively inhibiting binding of lipoprotein to the scavenger receptor protein type BI which selectively binds to low density lipoprotein and to modified lipoprotein having the characteristics of acetylated low density lipoprotein under conditions wherein the low density lipoprotein is bound to the scavenger receptor.

50. A method for screening patients for abnormal scavenger receptor protein activity or function comprising

determining the presence of a scavenger receptor protein selected from the group consisting of scavenger receptor protein type BI which selectively binds to low density lipoprotein and to modified lipoprotein having the characteristics of acetylated low density lipoprotein and scavenger receptor protein type CI which selectively binds to modified lipoprotein having the characteristics of acetylated low density lipoprotein and having the structure of the scavenger receptor protein defined by Sequence ID No. 6 in a patient sample, and comparing the scavenger receptor for to determine if the quantity present or the function of the receptor is equivalent to that present in normal cells.

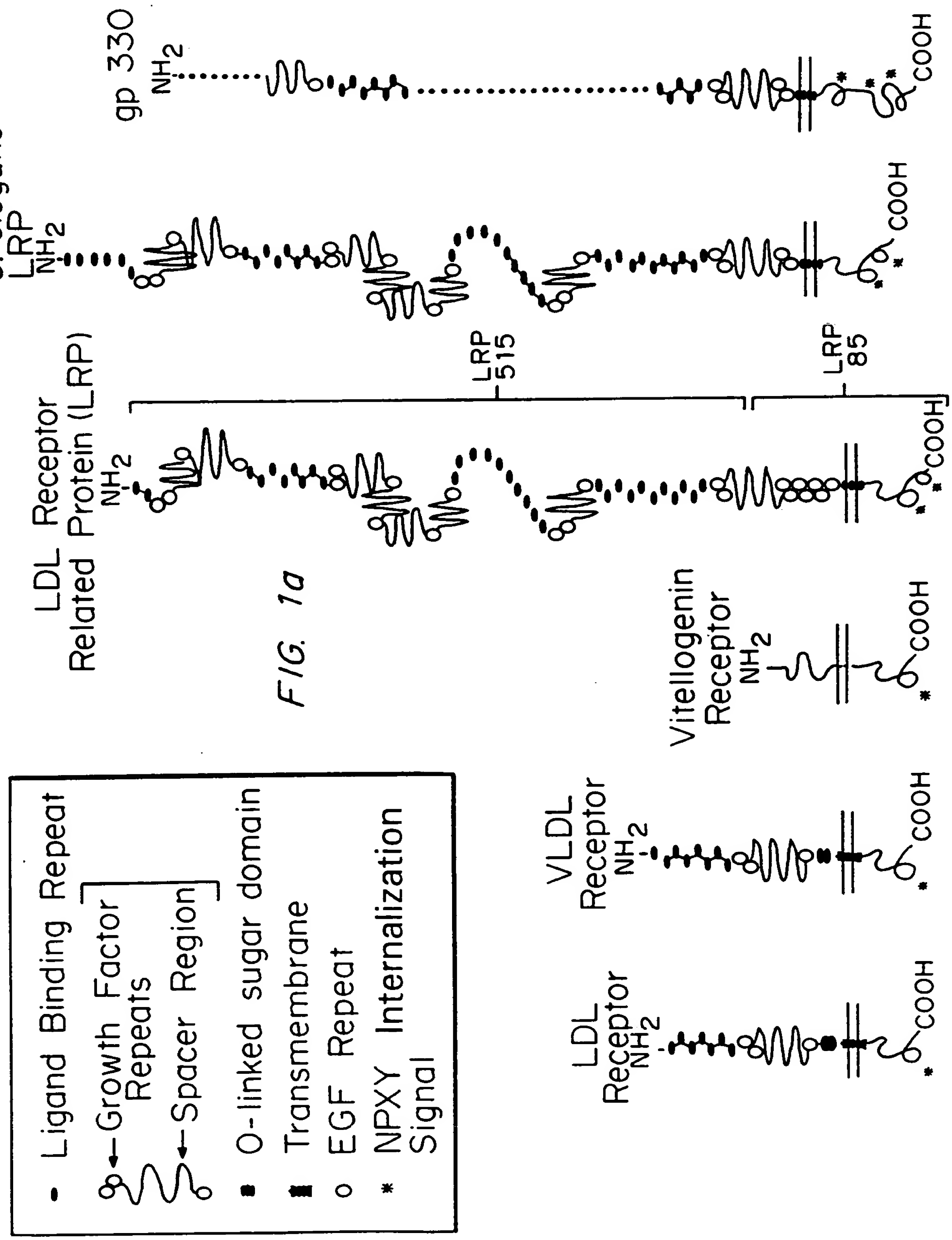

gp 330
NH_2
COOH
C. elegans
LRP
NH_2
COOH
LDL Receptor
Related Protein (LRP)
NH_2
LRP
515
LRP
85
COOH
Vitellogenin
Receptor
NH_2
COOH
VLDL
Receptor
NH_2
COOH
LDL
Receptor
NH_2
COOH
Ligand Binding Repeat
Growth Factor
Repeats
Spacer Region
O-linked sugar domain
Transmembrane
EGF Repeat
NPXY Internalization
Signal

FIG. 1a

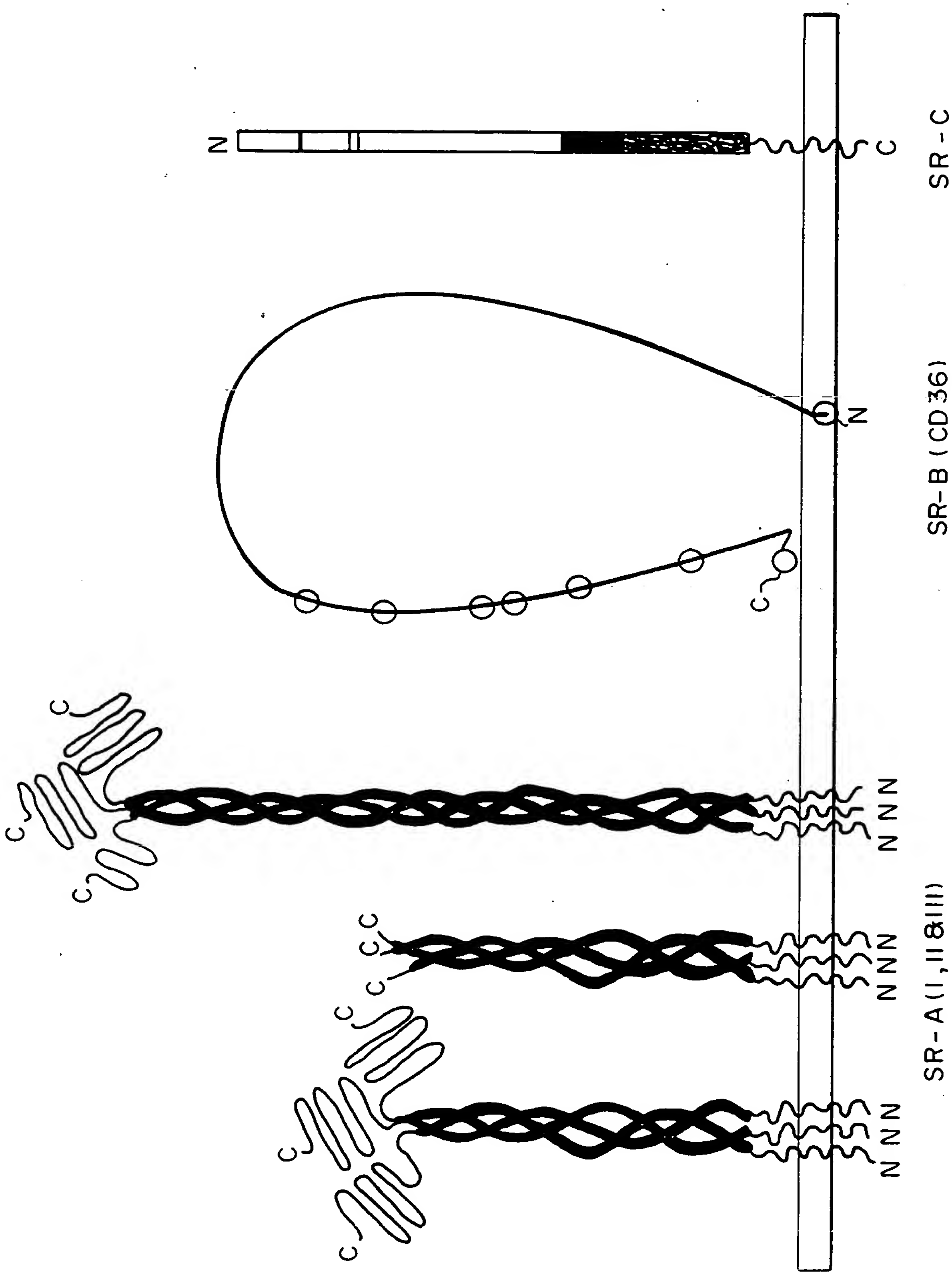


FIG. 1b

FIG. 2a

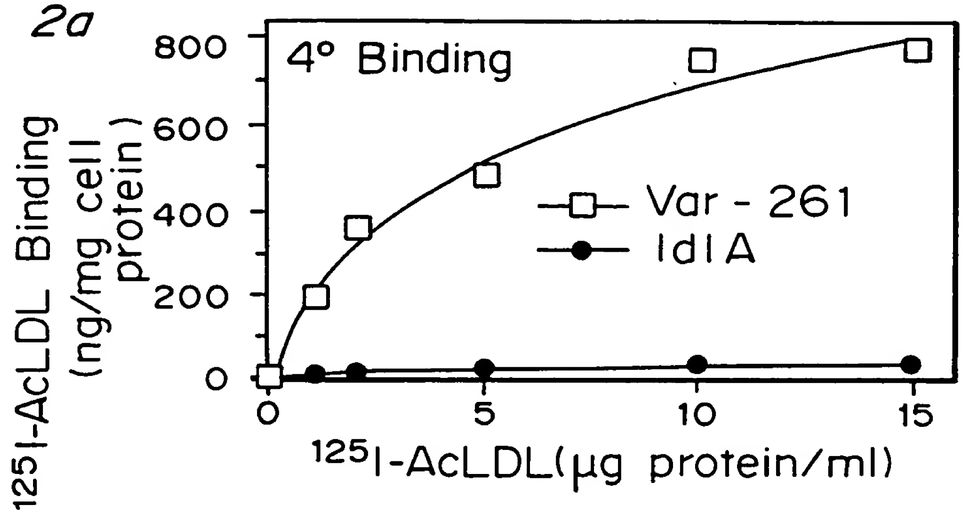


FIG. 2b

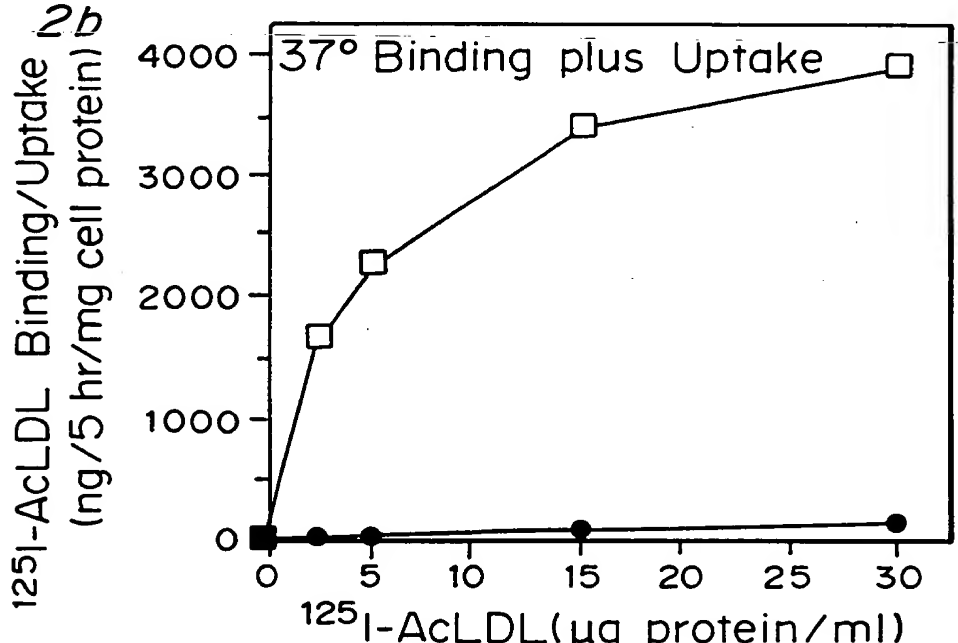
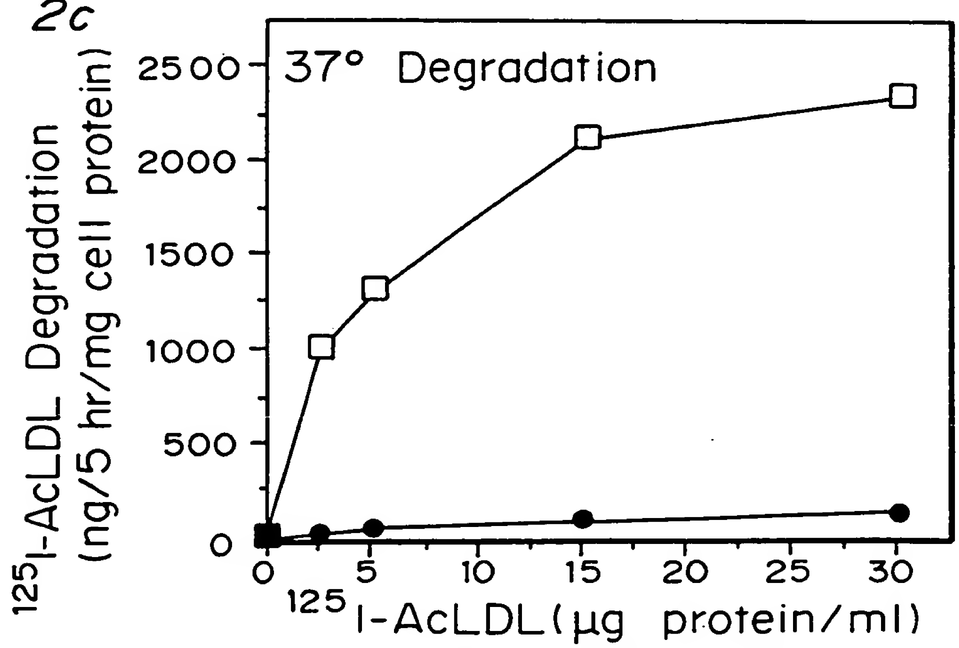


FIG. 2c



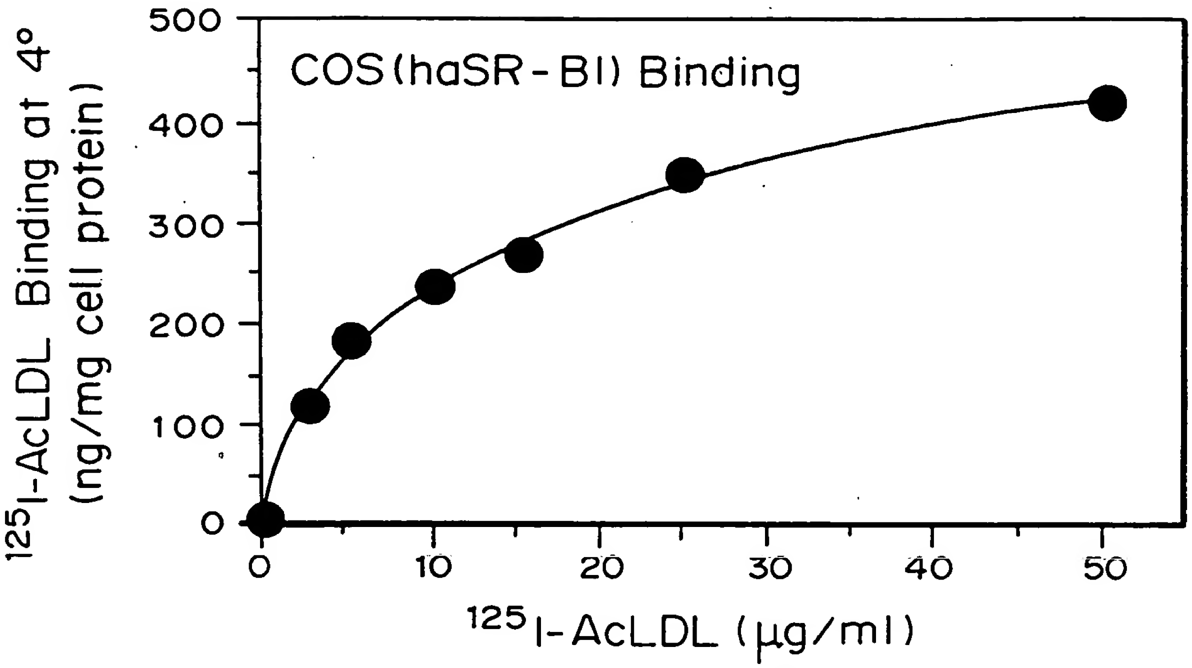

COS (haSR-BI) Binding
^{125}I-AcLDL Binding at 4°
(ng/mg cell protein)
500
400
300
200
100
0
0
10
20
30
40
50
^{125}I-AcLDL (µg/ml)

FIG. 3a

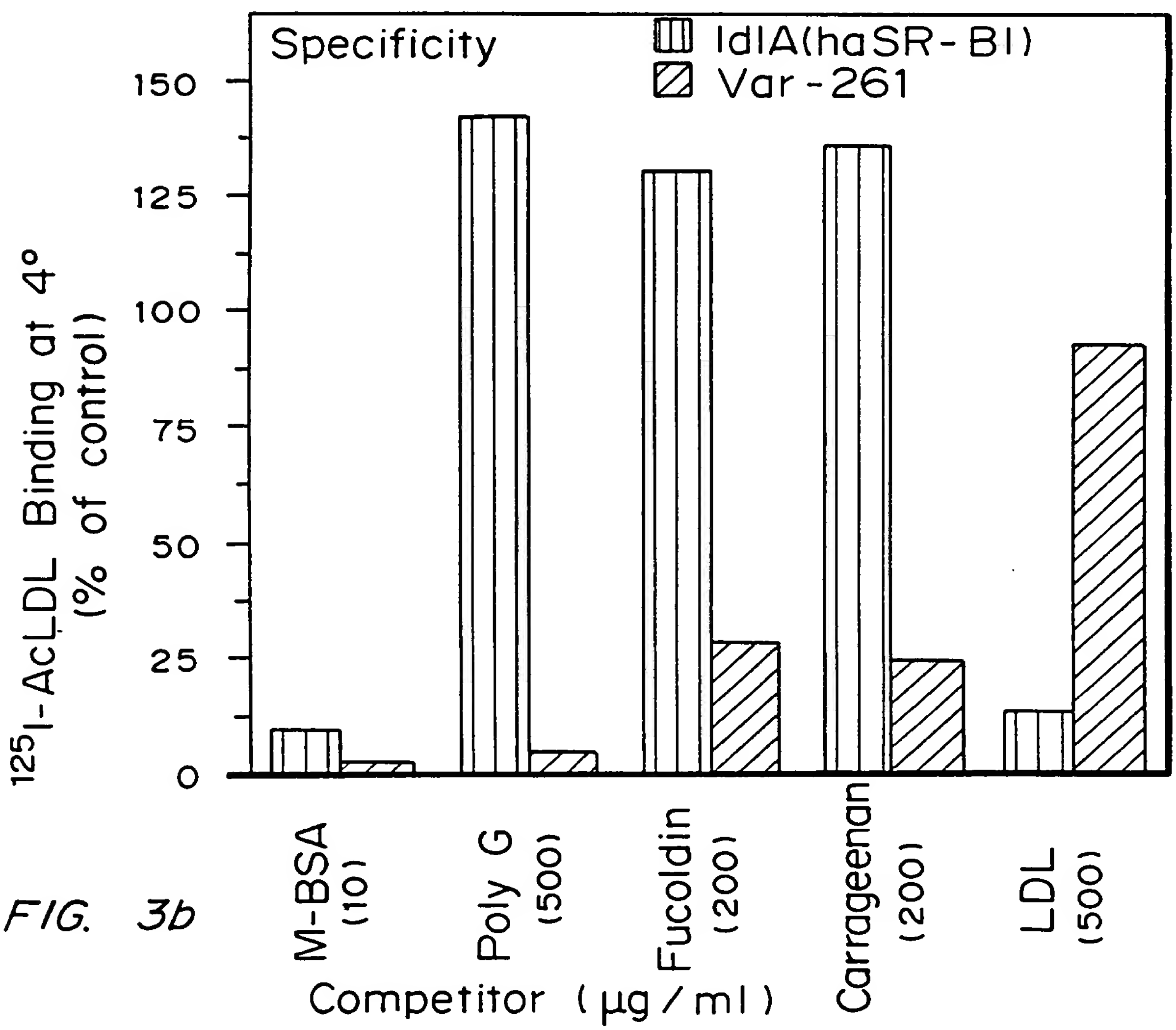

Specificity
ldlA(haSR-BI)
Var-261
^{125}I-AcLDL Binding at 4°
(% of control)
150
125
100
75
50
25
0
M-BSA (10)
Poly G (500)
Fucoidin (200)
Carrageenan (200)
LDL (500)
Competitor (µg/ml)

FIG. 3b

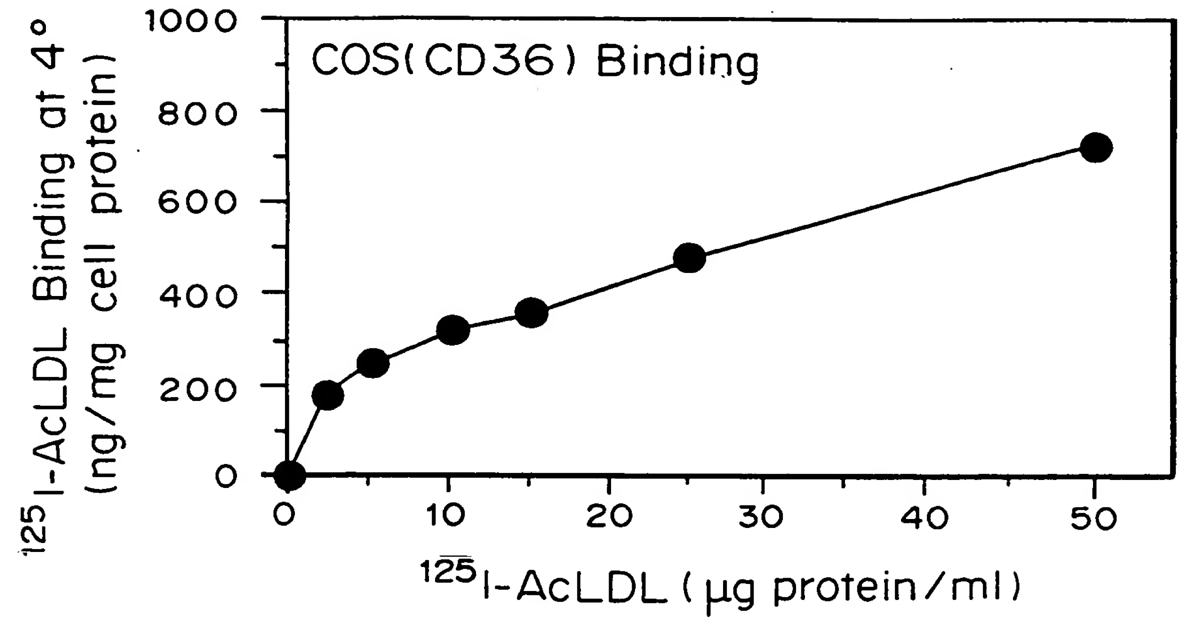

COS(CD36) Binding
^{125}I-AcLDL Binding at 4°
(ng/mg cell protein)
1000
800
600
400
200
0
0
10
20
30
40
50
^{125}I-AcLDL (μg protein/ml)

FIG. 4a

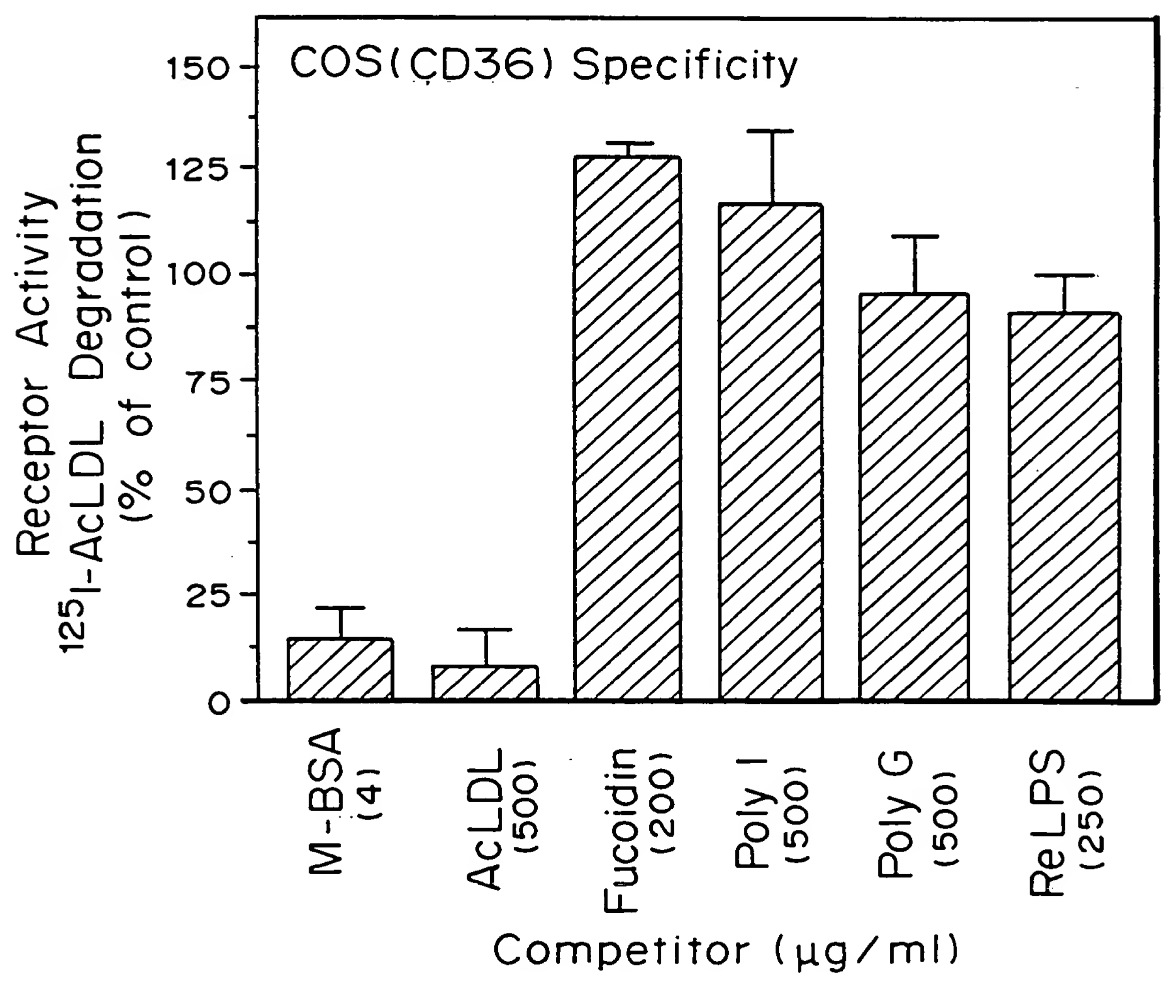

COS(CD36) Specificity
Receptor Activity
^{125}I-AcLDL Degradation
(% of control)
150
125
100
75
50
25
0
M-BSA (4)
AcLDL (500)
Fucoidin (200)
Poly I (500)
Poly G (500)
ReLPS (250)
Competitor (μg/ml)

FIG. 4b

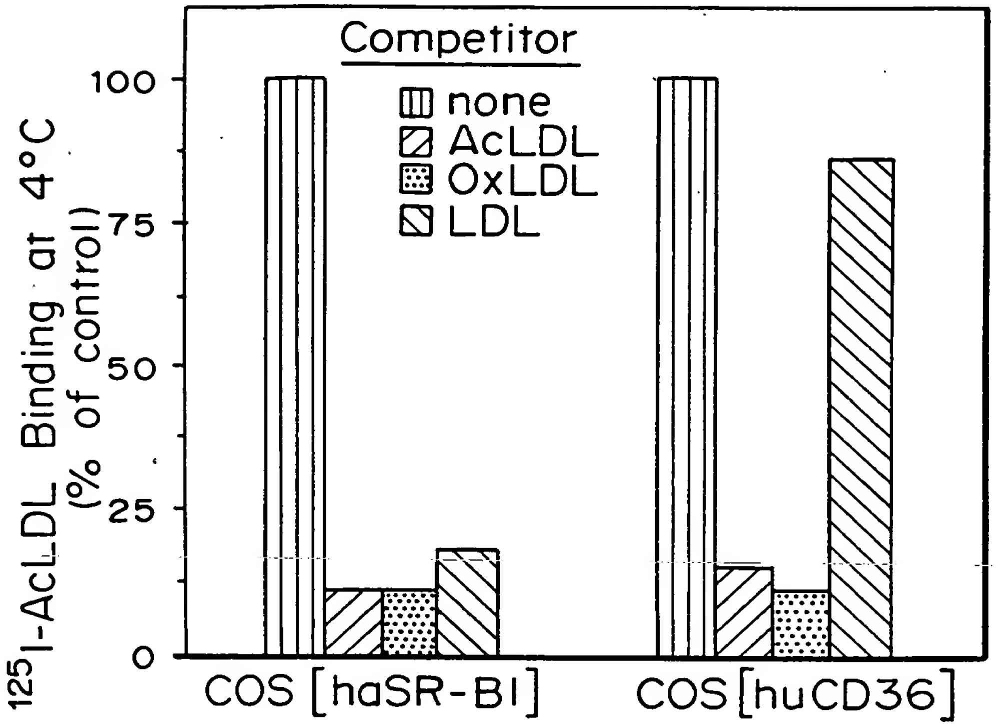

Competitor
none
AcLDL
OxLDL
LDL
100
75
50
25
0
^{125}I-AcLDL Binding at 4°C
(% of control)
COS [haSR-BI]
COS [huCD36]

FIG. 5

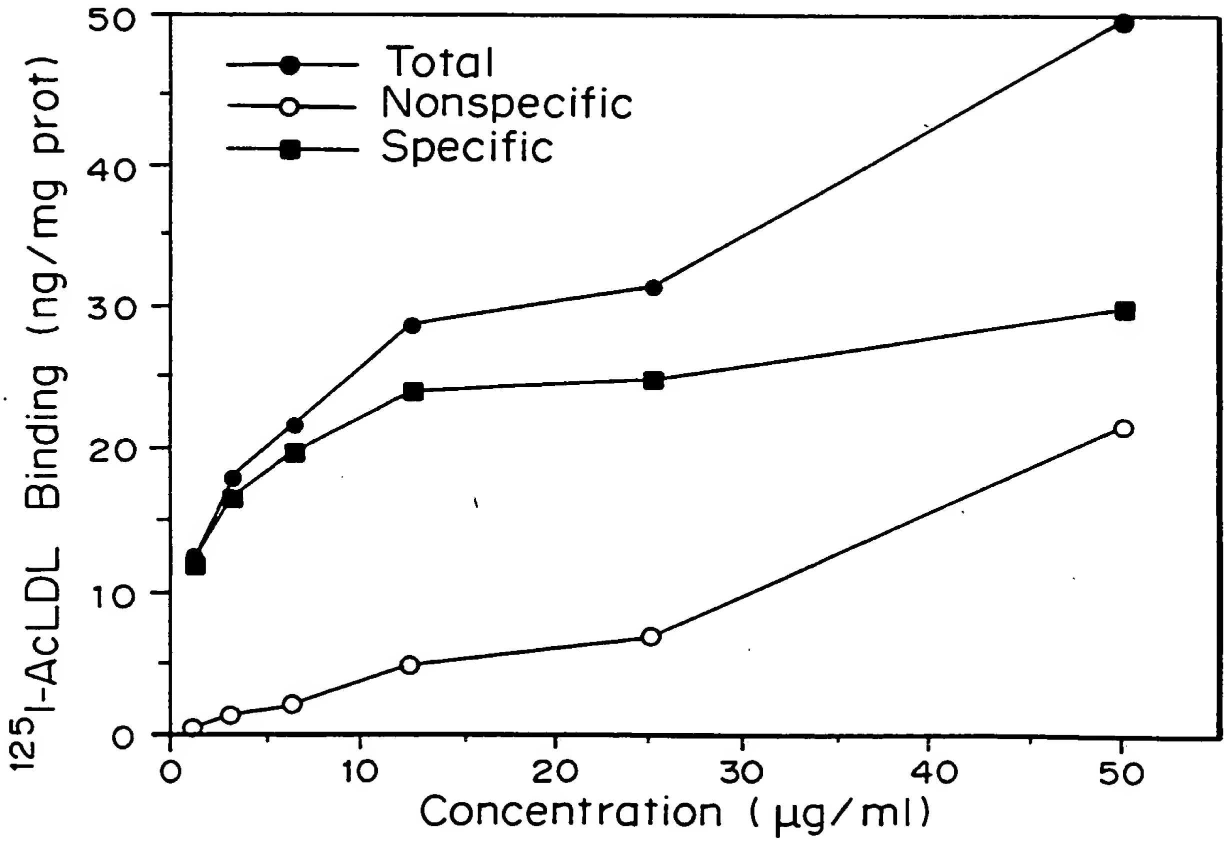

Total
Nonspecific
Specific
50
40
30
20
10
0
^{125}I-AcLDL Binding (ng/mg prot)
0
10
20
30
40
50
Concentration (μg/ml)

FIG. 6a

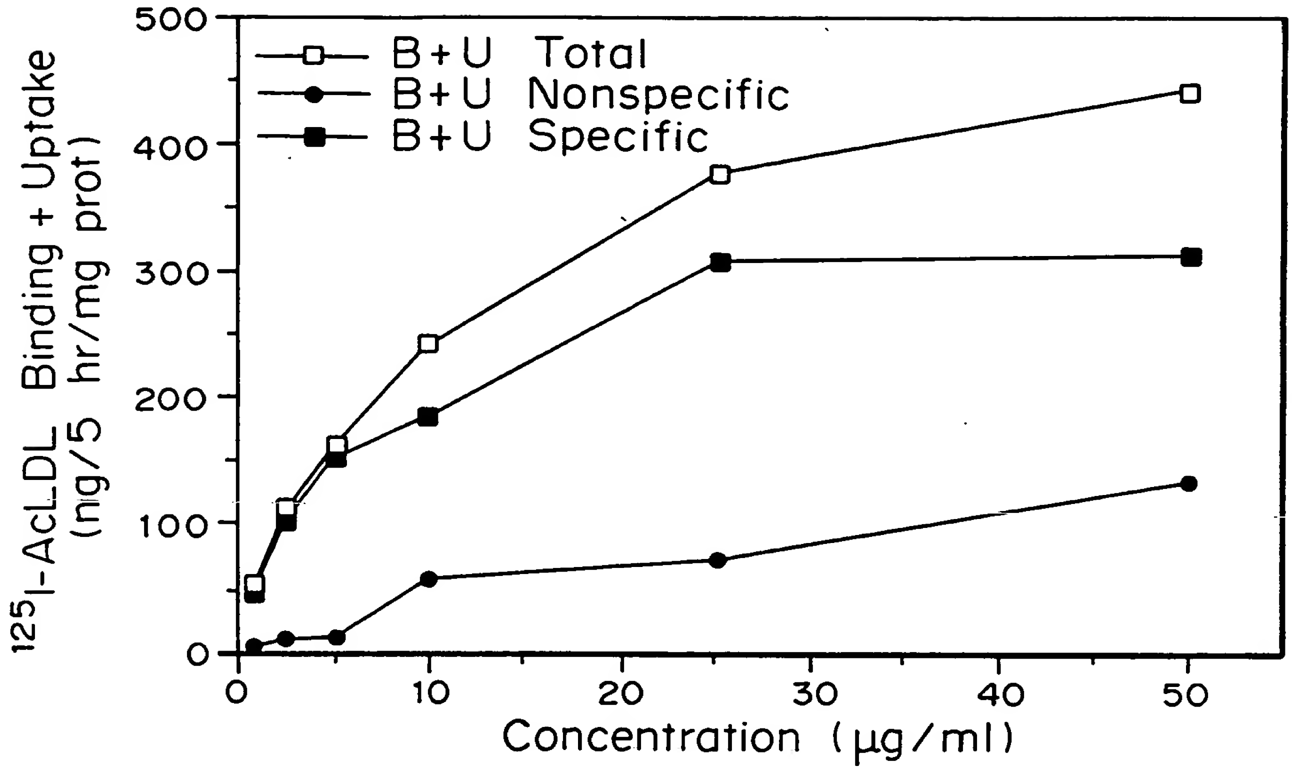

500
400
300
200
100
0
^{125}I-AcLDL Binding + Uptake
(ng/5 hr/mg prot)
B+U Total
B+U Nonspecific
B+U Specific
0
10
20
30
40
50
Concentration (μg/ml)

FIG. 6b

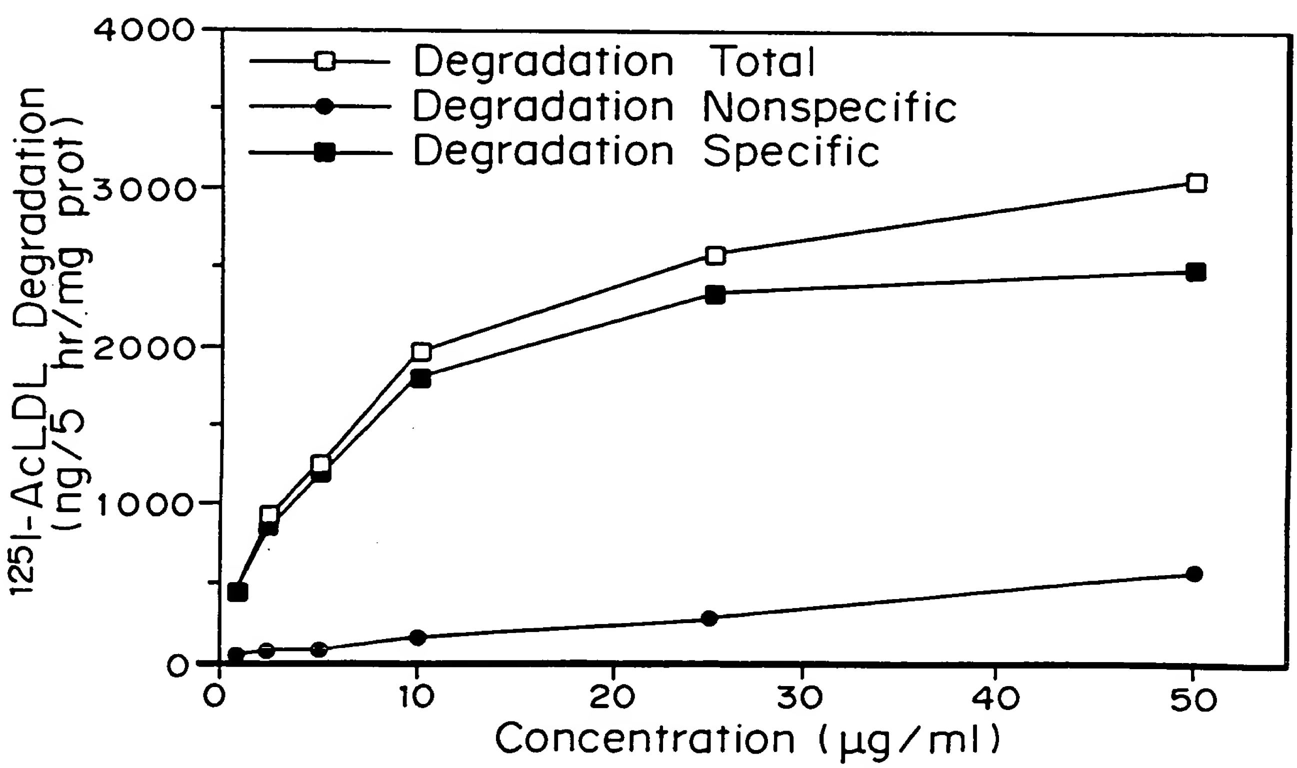

4000
3000
2000
1000
0
^{125}I-AcLDL Degradation
(ng/5 hr/mg prot)
Degradation Total
Degradation Nonspecific
Degradation Specific
0
10
20
30
40
50
Concentration (μg/ml)

FIG. 6c

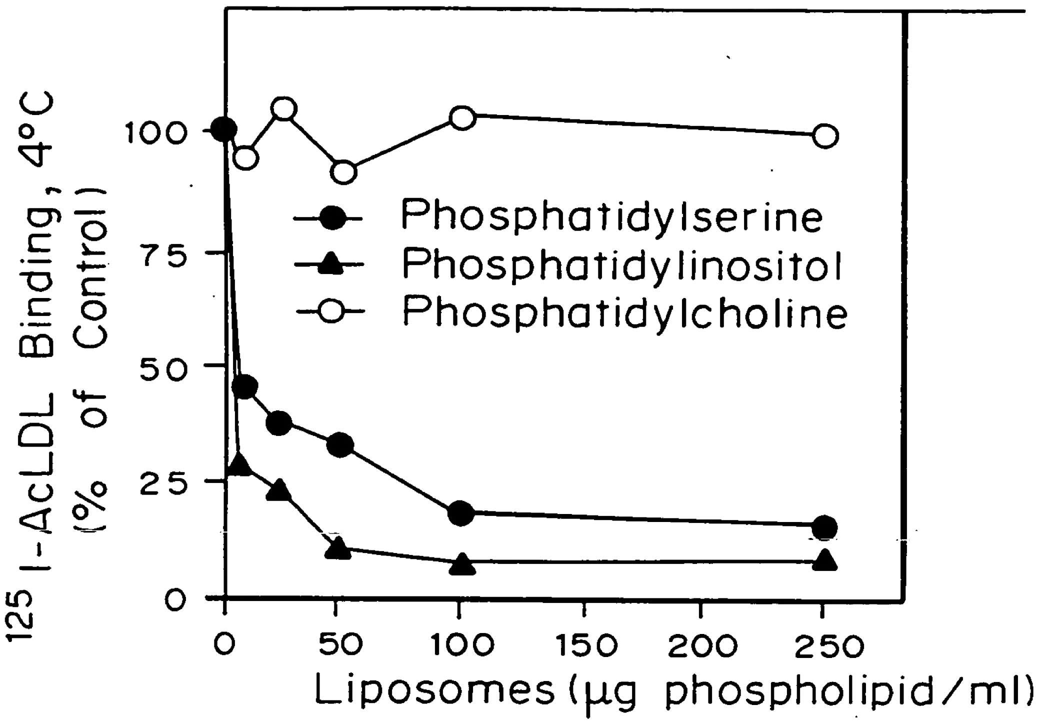

^{125}I-AcLDL Binding, 4°C (% of Control)
100
75
50
25
0
Phosphatidylserine
Phosphatidylinositol
Phosphatidylcholine
0
50
100
150
200
250
Liposomes (µg phospholipid/ml)

FIG. 7a

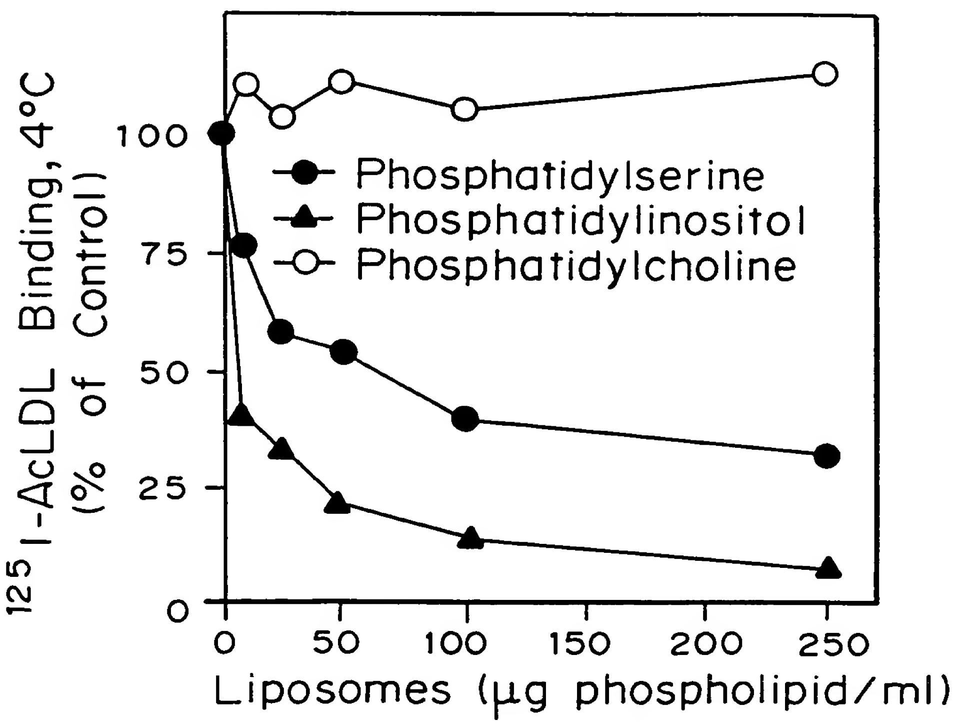

^{125}I-AcLDL Binding, 4°C (% of Control)
100
75
50
25
0
Phosphatidylserine
Phosphatidylinositol
Phosphatidylcholine
0
50
100
150
200
250
Liposomes (µg phospholipid/ml)

FIG. 7b

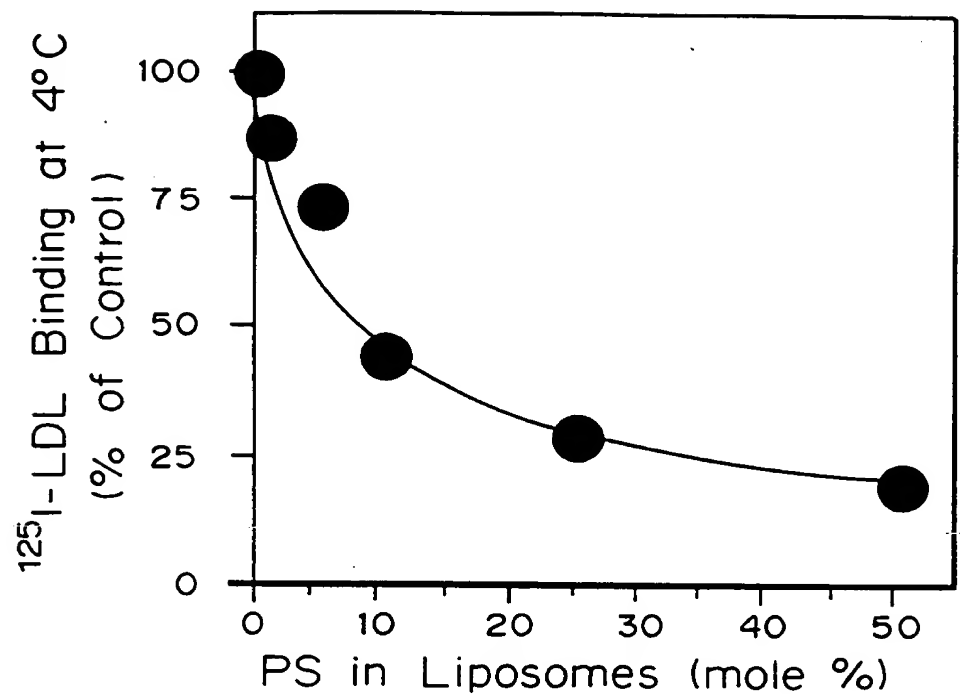

^{125}I-LDL Binding at 4°C
(% of Control)
100
75
50
25
0
0
10
20
30
40
50
PS in Liposomes (mole %)

FIG. 7c

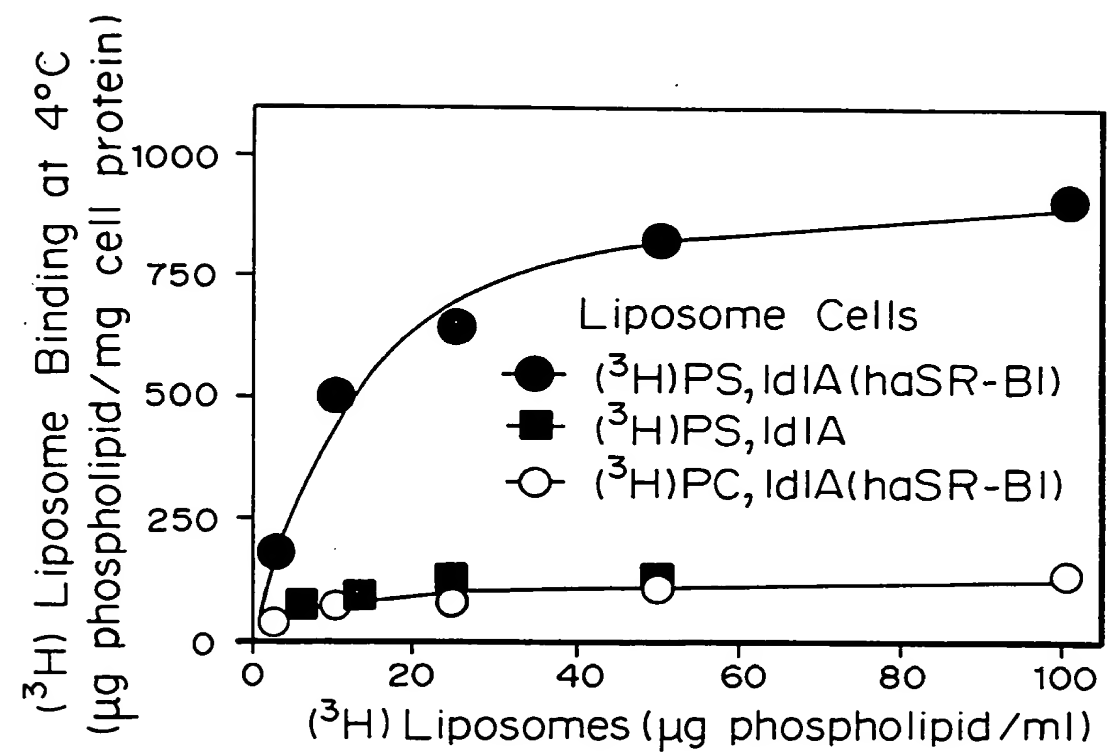
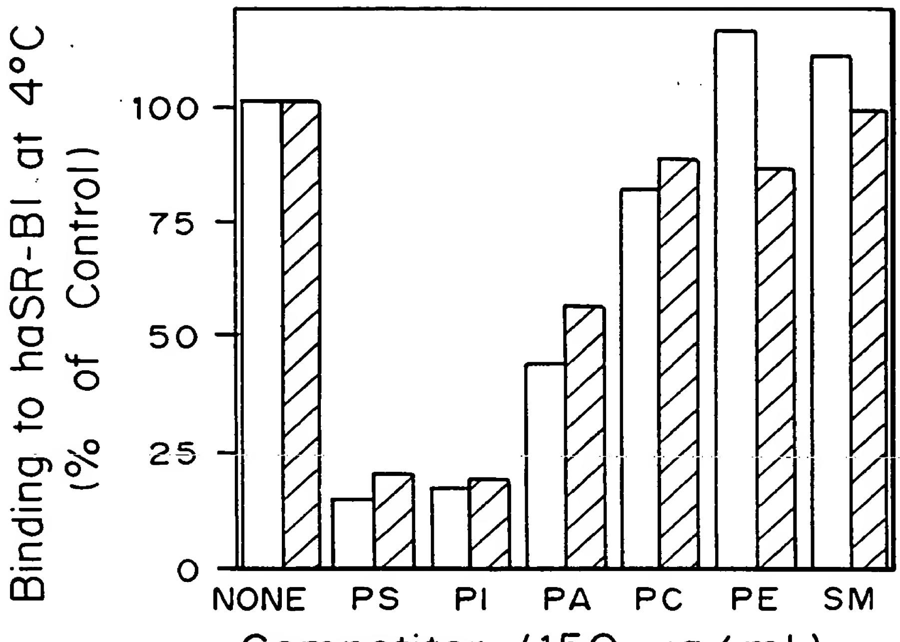

(^{3}H) Liposome Binding at 4°C
(µg phospholipid/mg cell protein)
1000
750
500
250
0
0
20
40
60
80
100
(^{3}H) Liposomes (µg phospholipid/ml)
Liposome Cells
(^{3}H)PS, ldlA(haSR-BI)
(^{3}H)PS, ldlA
(^{3}H)PC, ldlA(haSR-BI)

FIG. 7d



(^{3}H)PS Binding

^{125}I-LDL Binding

FIG. 7e

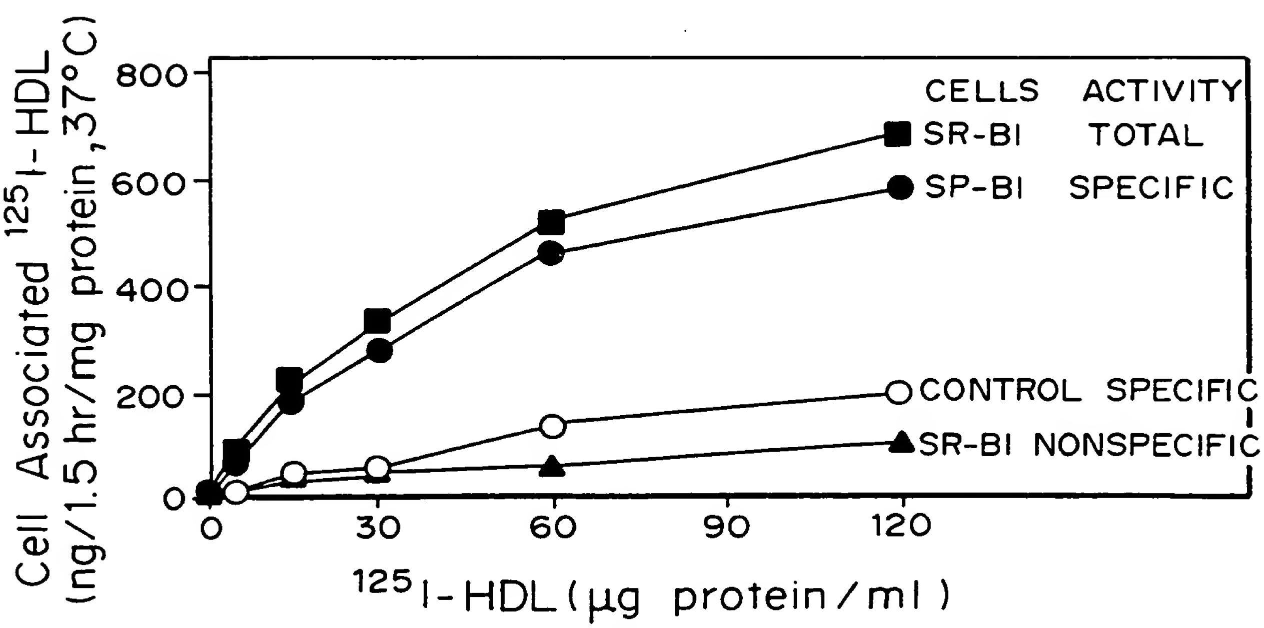


FIG. 8a

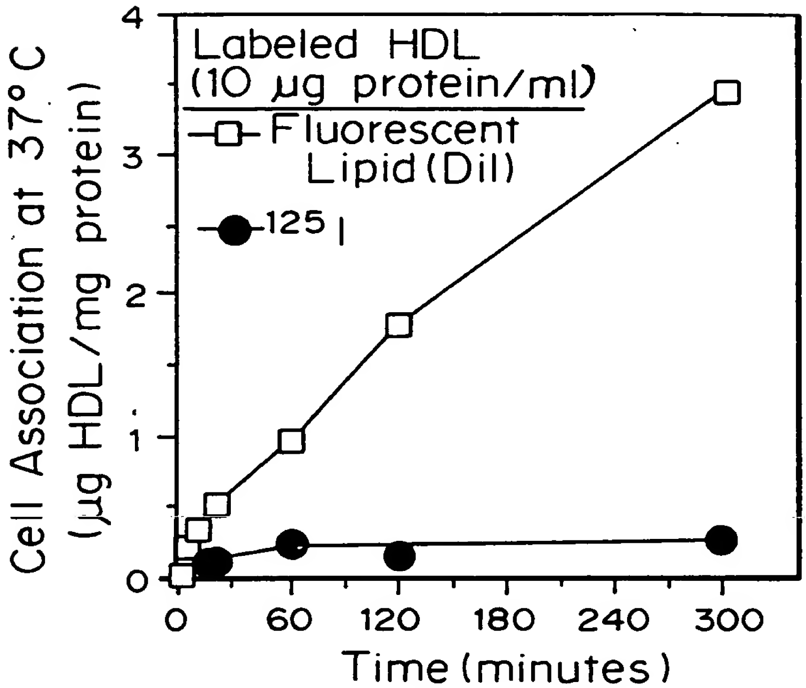

Cell Association at 37° C
(µg HDL/mg protein)
Labeled HDL
(10 µg protein/ml)
Fluorescent Lipid (DiI)
125 I
Time (minutes)
0
1
2
3
4
0
60
120
180
240
300

FIG. 8b

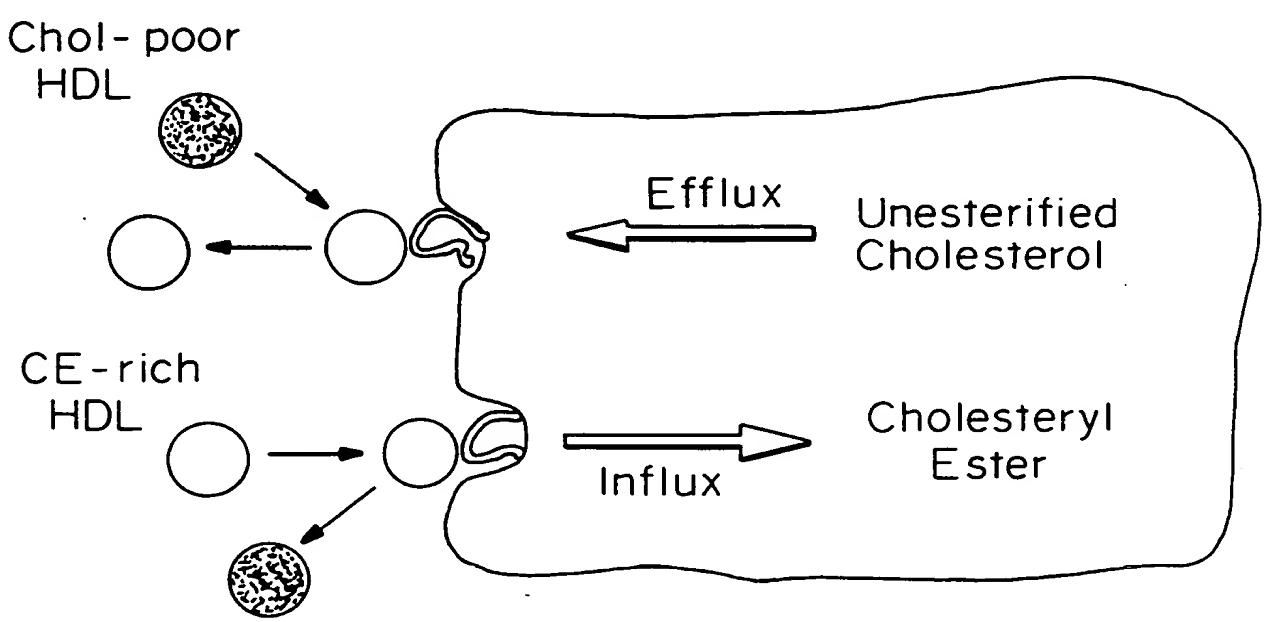

Chol- poor HDL
CE-rich HDL
Efflux
Unesterified Cholesterol
Cholesteryl Ester
Influx

FIG. 9

PCT

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/12, C07K 14/705, 16/28, A61K 38/17, C12Q 1/68, G01N 33/53 // C12N 15/11, C07H 21/04	A3	(11) International Publication Number: WO 96/00288 (43) International Publication Date: 4 January 1996 (04.01.96)

(21) International Application Number: PCT/US95/07721

(22) International Filing Date: 19 June 1995 (19.06.95)

(30) Priority Data:
265,428 23 June 1994 (23.06.94) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/265,428 (CIP)
Filed on 23 June 1994 (23.06.94)

(71) Applicant (*for all designated States except US*): MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US/US]; 77 Massachusetts Avenue, Cambridge, MA 02139 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): KRIEGER, Monty [US/US]; 139 Woodbine Circle, Needham, MA 02194 (US). ACTON, Susan, L. [US/US]; Apartment 1, 165 Hudson Street, Somerville, MA 02144 (US). PEARSON, Alan, M. [US/US]; 57A Cherry Street, Somerville, MA 02144 (US). RIGOTTI, Attilio [CL/CL]; Apartment 601, 2000 Commonwealth Avenue, Brighton, MA 02135 (US).

(74) Agent: PABST, Patrea, L.; Arnall Golden & Gregory, 2800 One Atlantic Center, 1201 West Peachtree Street, Atlanta, GA 30309-3450 (US).

(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LT, LV, MD, MG, MN, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

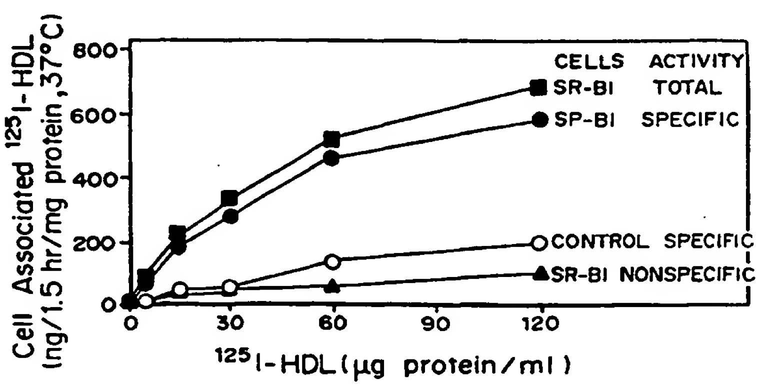
Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

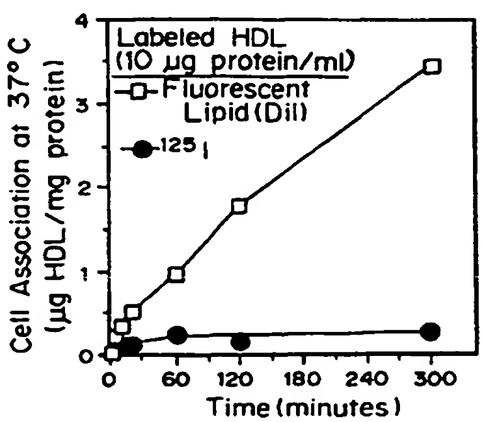
(88) Date of publication of the international search report: 4 April 1996 (04.04.1996)

(54) Title: CLASS BI AND CI SCAVENGER RECEPTORS

(57) Abstract

Two distinct scavenger receptor type proteins having high affinity for modified lipoproteins and other ligands have been isolated, characterized and cloned. HaSR-BI, an AcLDL and LDL binding scavenger receptor, which is distinct from the type I and type II macrophage scavenger receptors, has been isolated and characterized and DNA encoding the receptor cloned from a variant of Chinese Hamster Ovary Cells, designated Var-261. dSR-CI, a non-mammalian AcLDL binding scavenger receptor having high ligand affinity and broad specificity, was isolated from *Drosophila melanogaster*. The isolated receptors are useful in screening for drugs that inhibit uptake of cholesterol in endothelial or adipose cells or macrophages, respectively. They are also useful as probes for the isolation of other lipoprotein receptors and in research the roles of these receptors.





INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/07721

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/705 C07K16/28 A61K38/17 C12Q1/68
G01N33/53 //C12N15/11,C07H21/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 33, 19 August 1994 page 21003-21009 S. ACTON ET AL. 'Expression Cloning of SR-BI, a CD36-related Class B Scavenger Receptor' see the whole document ---	1-8, 11-22
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY 270 (27). 1995. 16221-16224. ISSN: 0021-9258, RIGOTTI A ET AL 'The class B scavenger receptors SR-BI and CD36 are receptors for anionic phospholipids.' see the whole document ---	1,2,6,7
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
9 February 1996	01.03.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Hix, R

Form PCT/ISA/210 (second sheet) (July 1992)

INTERI ONAL SEARCH REPORT

In 'onal Application No
PCI/US 95/07721

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY 270 (4). 1995. 1921-1927. ISSN: 0021-9258, FUKASAWA M ET AL 'Chinese Hamster Ovary Cells Expressing a Novel Type of Acetylated Low Density Lipoprotein Receptor: Isolation and characterization.' see the whole document ---	1,2,6-8
P,X	JOURNAL OF IMMUNOLOGY 155 (1). 1995. 367-376. ISSN: 0022-1767, KOBZIK L 'Lung macrophage uptake of unopsonized environmental particulates. Role of scavenger-type receptors.' see the whole document ---	1,2,6,7
X	NATURE, vol. 343, 8 February 1990 pages 531-535, T. KODAMA ET AL. 'Type I macrophage scavenger receptor contains alpha-helical and collagen-like coiled coils' see the whole document ---	1,2,11, 12, 16-18, 20-22
X	J BIOL CHEM 268 (7). 1993. 4569-4572. CODEN: JBCHA3 ISSN: 0021-9258, KRIEGER M ET AL 'MOLECULAR FLYPAPER HOST DEFENSE AND ATHEROSCLEROSIS STRUCTURE BINDING PROPERTIES AND FUNCTIONS OF MACROPHAGE SCAVENGER RECEPTORS.' see the whole document ---	1,7,8, 11,16-19
X	JOURNAL OF CLINICAL INVESTIGATION 93 (5). 1994. 2014-2021. ISSN: 0021-9738, LUOMA J ET AL 'Expression of alpha-2-macroglobulin receptor low density lipoprotein receptor-related protein and scavenger receptor in human atherosclerotic lesions.' see the whole document ---	1,2,7
X	BIOCHEM. J. (1994), 304(1), 69-73 CODEN: BIJOAK;ISSN: 0264-6021, 1994 DE RIJKE, YOLANDA B. ET AL 'Binding characteristics of scavenger receptors on liver endothelial and Kupffer cells for modified low-density lipoproteins' see the whole document ---	1,2,7
X	WO,A,90 05748 (MASSACHUSETTS INST TECHNOLOGY) 31 May 1990 whole document in particular pages 13 and 23 ---	1,2,6,7, 9-12

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-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 017 no. 622 (C-1130) ,17 November 1993 & JP,A,05 192179 (CHUGAI PHARMACEUT CO LTD) 3 August 1993, see abstract ---	9
X	PATENT ABSTRACTS OF JAPAN vol. 016 no. 118 (C-0922) ,25 March 1992 & JP,A,03 290184 (CHUGAI PHARMACEUT CO LTD) 19 December 1991, see abstract ---	1,2,7,8, 11,12, 16-19, 21,22,48
A	PROC NATL ACAD SCI U S A 89 (21). 1992. 10375-10379. CODEN: PNASA6 ISSN: 0027-8424, ABRAMS J M ET AL 'MACROPHAGES IN DROSOPHILA EMBRYOS AND L2 CELLS EXHIBIT SCAVENGER RECEPTOR -MEDIATED ENDOCYTOSIS.' see the whole document ---	23-43
A	COLD SPRING HARBOR SYMPOSIA ON QUANTITATIVE BIOLOGY, vol. LVII, 1992 pages 605-609, M.KRIEGER ET AL. 'Molecular flypaper, atherosclerosis, and host defense: structure and function of the Macrophage Scavenger Receptor' see the whole document ---	23-43
A	DEVELOPMENT, vol. 108, 1990 pages 269-280, ULRIKE SWIDA ET AL. 'Glue protein genes in Drosophila virilis: their organization, developmental control of transcription and specific mRNA degradation' see the whole document -----	23-43

3

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 48-49
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although these claims (as far as in vivo methods are concerned) are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- claims 1-8,11-22,48, 49 and, partially, 9,10,44-47,50
- claims 23-43 and, partially, 9,10,44-47,50

See continuation sheet PCT/ISA/210

1. [X] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

- claims 1-8,11-22,48,49 and partially claims 9,10,44-47,50

  Scavenger receptor protein BI, nucleic acid sequence and antibodies. Methods of screening for scavenger receptor protein BI and removing low density lipoprotein from blood samples and inhibiting lipoprotein or lipid uptake by using or inhibiting the scavenger receptor protein BI.

- claims 23-43 and partially claims 9,10,44-47,50

  Scavenger receptor protein CI, nucleic acid sequence and antibodies. Methods of screening for scavenger receptor protein CI.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 95/07721

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9005748	31-05-90	EP-A- 0406367	09-01-91
		JP-T- 6500765	27-01-94

Annu. Rev. Biochem. 1999. 68:523–558
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CHARTING THE FATE OF THE "GOOD CHOLESTEROL": IDENTIFICATION AND CHARACTERIZATION OF THE HIGH-DENSITY LIPOPROTEIN RECEPTOR SR-BI

Monty Krieger
Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; email: krieger@mit.edu

Key Words lipoprotein, liver, steroidogenic tissue, selective uptake, lipid transport, atherosclerosis, hormonal regulation, ovary, adrenal

■ **Abstract** Risk for cardiovascular disease due to atherosclerosis increases with increasing concentrations of low-density lipoprotein (LDL) cholesterol and is inversely proportional to the levels of high-density lipoprotein (HDL) cholesterol. The receptor-mediated control of plasma LDL levels has been well understood for over two decades and has been a focus for the pharmacologic treatment of hypercholesterolemia. In contrast, the first identification and characterization of a receptor that mediates cellular metabolism of HDL was only recently reported. This receptor, called scavenger receptor class B type I (SR-BI), is a fatty acylated glycoprotein that can cluster in caveolae-like domains on the surfaces of cultured cells. SR-BI mediates selective lipid uptake from HDL to cells. The mechanism of selective lipid uptake is fundamentally different from that of classic receptor-mediated endocytic uptake via coated pits and vesicles (e.g. the LDL receptor pathway) in that it involves efficient receptor-mediated transfer of the lipids, but not the outer shell proteins, from HDL to cells. In mice, SR-BI plays a key role in determining the levels of plasma HDL cholesterol and in mediating the regulated, selective delivery of HDL-cholesterol to steroidogenic tissues and the liver. Significant alterations in SR-BI expression can result in cardiovascular and reproductive disorders. SR-BI may play a similar role in humans; thus, modulation of its activity may provide the basis of future approaches to the treatment and prevention of atherosclerotic disease.

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Receptor-Mediated Endocytosis 525
Physiologic, Genetic, and Pharmacologic Regulation 527

0066-4154/99/0701-0523$08.00

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INTRODUCTION

The circulatory system sustains the life of individual cells by providing nutrients and removing waste, as well as transporting signals (e.g. hormones) that help coordinate and integrate the metabolism of cells in diverse tissues. The intercellular transport of critical components is well regulated, and its disruption can result in disease. For example, aberrant lipid transport can result in atherosclerosis, the major cause of heart disease and stroke, which together represent the number-one killer in the United States (1). It is widely appreciated that cholesterol plays an important role in atherogenesis. Normally, most cholesterol serves as a structural element in the walls of cells, while much of the rest is in transit through the blood or functions as the substrate for the synthesis of bile acids in the liver, steroid hormones in endocrine tissue (e.g. adrenal gland, ovary, and testis), or vitamin D in skin.

The intercellular transport of unesterified ("free") cholesterol, cholesteryl esters, and other lipids, including triglycerides and certain vitamins, through the aqueous circulatory system is dependent on the packaging of these hydrophobic molecules into water-soluble carriers called lipoproteins. Most lipoprotein particles comprise protein and phospholipid shells surrounding a core of neutral lipid. There are four major classes of mammalian lipoproteins, some of which are named based on their differing buoyant densities (2). They are low-density lipoprotein (LDL), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL, principally a triglyceride carrier synthesized by the liver), and chylomicrons (dietary lipid carrier synthesized in the intestines).

These lipoproteins have different protein and lipid compositions, sizes, and physiological and pathophysiological activities (2). LDL has a hydrophobic core of approximately 1700 cholesteryl ester molecules and an outer phospholipid and cholesterol monolayer shell containing a single copy of the very large pro-

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tein called apolipoprotein B-100 (3, 4); however, the size and density of LDL particles can vary (5). HDL particles are smaller and more heterogeneous than LDL particles. The major apolipoproteins of HDL are apoA-I and apoA-II (with the minor components apoA-IV, the apoCs, apoD, apoE, and others) (2, 6–9). The enzyme paroxonase can be found on some HDL particles and has recently been implicated in xenobiotic metabolism and atherosclerosis in an animal model (10). HDLs are classified into two broad, interconverting groups, α-HDLs, which are larger particles with a core of neutral lipid, and pre-β-HDLs, which have little neutral lipid (11, 12). The pathways of the biosynthesis of lipoproteins are complex and are currently the subject of investigation. Their production can involve not only the assembly of apolipoproteins and lipids in the secretory apparatus of hepatic and other cells, but also the extracellular modification or interconversion of lipoproteins, including the transfer of apolipoprotein and lipid components (2, 6–9, 13, 14). For example, after hepatic secretion of VLDL, it is converted to intermediate-density lipoprotein (IDL) and eventually LDL (Figure 1). A number of key enzymes participate in these transformations (2, 15, 16), including lipoprotein lipase (LPL), hepatic lipase, lecithin:cholesterol acyltransferase, and cholesteryl ester transfer protein (CETP). Perhaps the most striking difference in the function of lipoproteins in cholesterol transport and physiology is the differing effects of plasma LDL and HDL cholesterol concentrations on the relative risk for coronary artery disease. There is a direct correlation of risk with plasma LDL cholesterol concentration and an inverse correlation with plasma HDL cholesterol concentration (17–20).

For effective cholesterol transport and cellular cholesterol homeostasis to occur, lipoproteins are targeted by cell surface receptors to appropriate cells in tissues to deliver or remove cholesterol. Such targeting should be regulated to ensure that intercellular cholesterol transport is coordinated with intracellular cholesterol metabolism. The receptor-mediated control of plasma LDL levels has been well-defined by the elegant work of Brown, Goldstein, and colleagues (20–22), and very recent studies have now provided new insights into receptor-mediated HDL metabolism (14, 23–27; see below). After a brief review of the LDL receptor pathway of receptor-mediated endocytosis, this review focuses on the structure and function of the HDL receptor scavenger receptor class B type I (SR-BI).

LDL RECEPTOR PATHWAY

Receptor-Mediated Endocytosis

Under normal conditions, LDL receptors are responsible for the removal of much of the LDL from the circulation. The current view of the structure of the LDL receptor is based on its primary sequence as well as biochemical and biophysical analyses (22, 28–31). The LDL receptor glycoprotein is a mosaic of five distinct, relatively common motifs found in a variety of proteins. These

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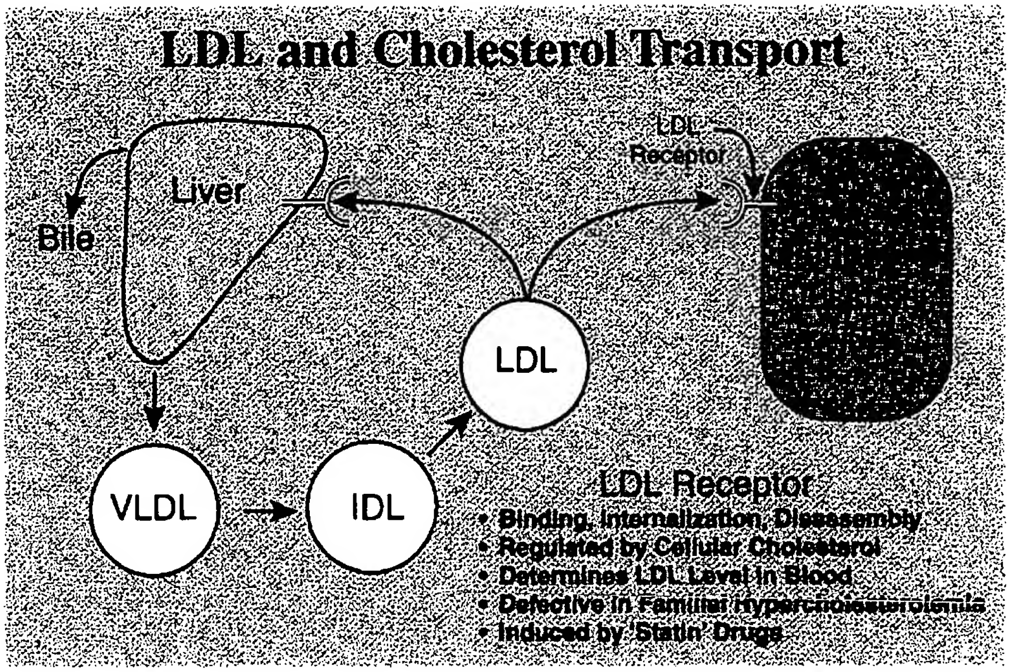

Figure 1 Low-density lipoprotein (LDL) and the LDL receptor in cholesterol transport. The liver secretes triglyceride-rich very-low-density lipoprotein (VLDL), which is converted in the plasma to intermediate-density lipoprotein (IDL) and then cholesteryl ester-rich LDL. Plasma LDL cholesterol levels are controlled by the receptor-mediated endocytic clearance of LDL from the circulation by LDL receptors. These receptors are most abundantly expressed in the liver, but are also found in many other tissues (see text for details). (From Reference 14.)

include the epidermal growth factor (EGF) precursor and several complement components (e.g. component C9). LDL binds to a group of Ca^{2+}-dependent (29–31), complement-related, cysteine-rich domains at the extracellular N-terminus of the molecule. During the biosynthesis and transport of LDL receptors through the secretory pathway (Figure 2*A*), a cluster of serine/threonine O-linked oligosaccharide chains is added to the extracellular portion of the receptor near the plasma membrane. These sugars protect the receptor from cleavage by proteases (32, 33). The majority of the LDL receptors in the body are on the surfaces of hepatocytes, although the cells of virtually all other tissues (peripheral tissues) express some LDL receptors (Figure 1; see 20–22 for reviews). LDL receptors, located in coated pits on the surfaces of cells (Figure 2*A*), bind LDL specifically and with high affinity. After binding, the receptor-lipoprotein complex is internalized via coated pits and coated vesicles, which are then converted into endosomes. The low pH in the lumen of endosomes induces the dissociation of LDL from its receptor, freeing the receptor so that it can recycle to the surface and participate in additional rounds of LDL endocytosis. The receptor-free, entire

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LDL particle is then delivered to lysosomes, wherein it is degraded by enzymatic hydrolysis, releasing the core cholesterol for subsequent cellular metabolism (Figure 2A). This whole-particle uptake pathway is called "receptor-mediated endocytosis" (21) and, as indicated below, is strikingly different from the major mechanism by which HDL delivers cholesterol to cells.

Physiologic, Genetic, and Pharmacologic Regulation

Cholesterol-mediated feedback regulation of the levels of both LDL receptors and cellular cholesterol biosynthesis helps ensure cellular cholesterol homeostasis (20–22). For example, reduction of the intracellular cholesterol pool by conversion of cholesterol to bile acids (liver) or steroid hormones (endocrine tissue) results in stimulation of LDL receptor synthesis, increased LDL endocytosis, and thus replenishment of the cellular cholesterol pool. Substantial progress has been made in identifying the promoter regulatory elements, membrane-bound transcription factors [sterol regulatory element-binding protein (SREBP)], and other components of the cholesterol-sensing/regulatory system for the transcriptional regulation of LDL receptor activity (34). A variety of mutations in the structural gene for the LDL receptor in humans result in familial hypercholesterolemia, a disease characterized by elevated plasma LDL cholesterol and premature atherosclerosis (20). A major breakthrough in the pharmacologic treatment of hypercholesterolemia has been the development of the "statin" drugs, which are HMG-CoA reductase inhibitors (35). HMG-CoA reductase is the rate-controlling enzyme in cholesterol biosynthesis, and its inhibition in the liver stimulates LDL receptor expression. As a consequence, both plasma LDL cholesterol levels and the risk for atherosclerosis decrease.

HDL METABOLISM AND SELECTIVE LIPID UPTAKE

HDL has been the subject of intense study for decades, and much has been learned, especially about its constituent parts and its dynamic remodeling in the plasma (2, 6–9, 15, 16, 36–38). In rodents, in which HDL transports most of the plasma cholesterol, HDL has been shown to be an important source of cholesterol for biliary excretion and steroidogenesis (39–48). HDL appears to originate as neutral lipid-poor discoidal particles secreted by the liver and intestine, which are converted in the plasma to spherical particles with neutral lipid cores. Although in some rare genetic disorders changes in HDL concentrations can be explained by alterations in the rates of HDL synthesis, clearance rather than synthesis appears to be the major mechanism controlling plasma levels of HDL cholesterol and apoA-I (15, 36). Some studies have suggested that variations in the catabolism of apoA-I can occur as secondary consequences of primary alterations in HDL lipid catabolism (25, 49, 50).

HDL appears to function as a shuttle transferring cholesterol between cells and other lipoproteins (Figure 3). HDL is thought to remove "free" cholesterol

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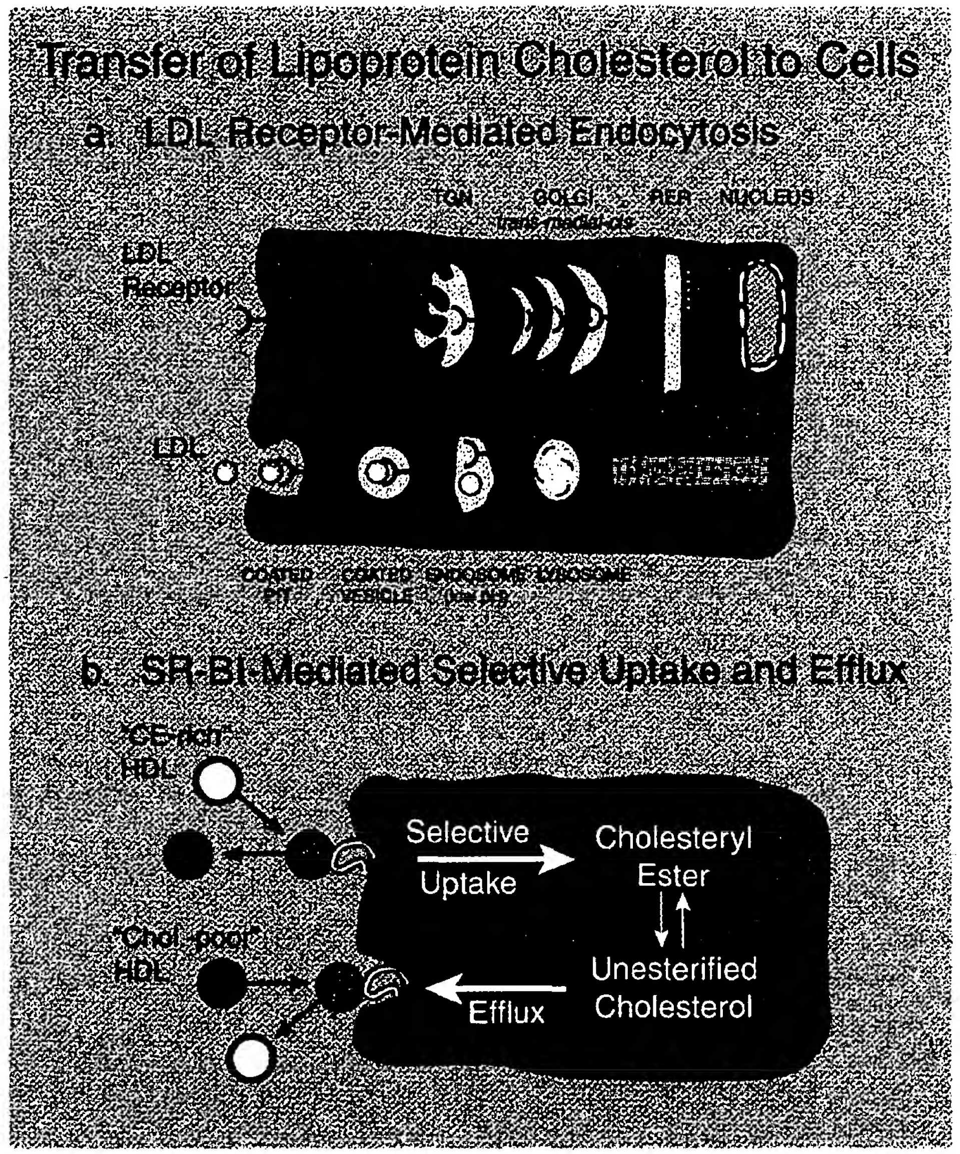

from peripheral tissues, after which much of the HDL-associated free cholesterol is converted to cholesteryl ester by the plasma enzyme lecithin:cholesterol acyl-transferase (6–9, 36–38). Subsequently, the cholesteryl esters in HDL can be transferred either directly to target cells or, in some species, to other lipoproteins by the plasma protein CETP for additional transport and metabolism (16). Studies in rodents have shown that the main tissues to which HDL delivers its cholesteryl esters are the liver [for secretion into the bile, bile acid synthesis, or packaging and secretion in VLDL (41, 51)] and steroidogenic tissues [for storage and hormone synthesis (14, 40, 42, 44, 46, 47, 52–54)]. For example, in the rat, the efficient delivery of cholesterol from intravenously infused HDL to steroidogenic tissues results in suppression of sterol synthesis (52, 55–57). Cultured ovar-

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Figure 2 Transfer of lipoprotein cholesterol to cells. (*a*) Low-density lipoprotein (LDL) receptors are synthesized as integral membrane proteins in the rough endoplasmic reticulum (RER) and are co- and post-translationally modified by asparagine-linked (N-linked) and serine/threonine-linked (O-linked) glycosylation when they are transported from the ER to the Golgi apparatus and transgolgi network (TGN). Subsequently they are delivered to the cell surface where they cluster in coated pits. Apolipoprotein B mediates the high-affinity binding of LDL to the receptor at the cell surface. Endocytosis begins with the invagination of coated pits containing the receptor-LDL complex. This results in the formation of coated endocytic vesicles, which are then converted to endosomes. The low pH in the lumen of the endosome induces receptor-LDL dissociation, with consequent recycling of the receptor to the cell surface and lysosomal disassembly of the LDL particle by hydrolytic enzymes. The cholesterol released from the core of the degraded LDL can then enter the cell's metabolic pool of cholesterol. (Modified from Reference 92.) (*b*) SR-BI binds high-density lipoprotein (HDL) with high affinity and then can facilitate the selective uptake of its cholesteryl esters. (*Upper*) Selective uptake does not involve lysosomal degradation of the lipoprotein particle; instead, the lipid-depleted particle is released from the cells (see text for details). (*Lower*) Studies with cultured cells have also shown that SR-BI can mediate the efflux of unesterified cholesterol from cells (see text). CE, cholesteryl ester

ian luteal cells from many species appear to require lipoprotein cholesterol for high-level steroidogenesis, and HDL has been proposed to be important in maintaining a high level of ovarian luteal cell steroidogenesis in cattle, sheep, pigs, rabbits, and rodents (58–62), although it may be less important than LDL for human ovarian steroidogenesis (63, 64). The overall pathway of HDL-mediated transport of cholesterol from extrahepatic tissues to the liver (Figure 3) is called reverse cholesterol transport (65). Reverse cholesterol transport is believed to play a critical role in cholesterol homeostasis and may be responsible for some of the protective effects of HDL against atherosclerosis (7–9, 38, 39).

In rodents and rabbits both in vitro and in vivo experiments have established that the primary mechanism of cholesteryl ester delivery from HDL to cells is fundamentally different from that of the LDL receptor pathway, because it does not involve endocytosis and degradation of the entire lipoprotein particle (38, 39, 42–48, 66–77). Instead, HDL binds to the cell surface and transfers its cholesteryl esters to the cell and then the lipid-depleted HDL dissociates from the cells and re-enters the circulation (Figures 2*B* and 3). This novel cellular mechanism for HDL cholesterol uptake is called selective lipid uptake (42, 44, 71; reviewed in 39). Both hepatic lipase (79, 80) and lipoprotein lipase (81) appear to stimulate selective uptake (also see below), and there may be differences in the efficiency of selective uptake depending on the composition of the HDL particles (82). The selective delivery of lipids, but not protein, from HDL to cells appears to be, at least in rodents, an important mechanism for the transport of cholesterol to steroidogenic tissues (adrenal gland, ovary, and testis) (40, 42–44, 46, 66, 67, 70–73, 78) and to the liver (38, 40, 42–45, 52, 68, 75). In rats, the liver clears

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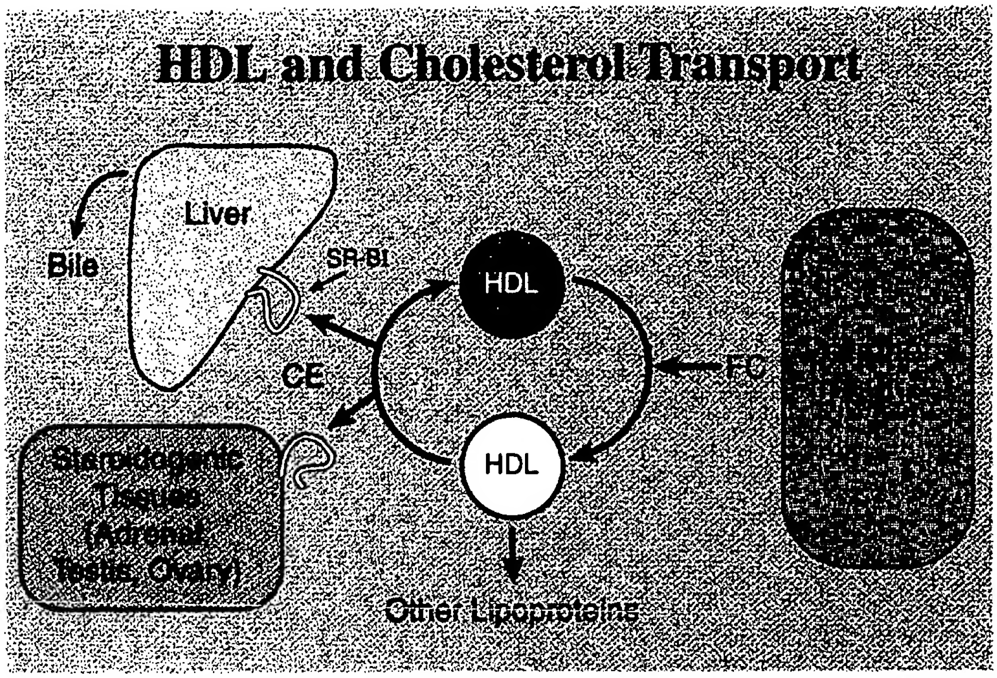

Figure 3 High-density lipoprotein (HDL) and the HDL receptor SR-BI in cholesterol transport. HDL appears to function as a shuttle for the movement of cholesterol through the body. It is proposed that HDL removes unesterified, or "free" cholesterol from peripheral tissues, after which much of the cholesterol is esterified [cholesteryl ester (CE)] by the plasma enzyme lecithin:cholesterol acyltransferase. Subsequently, HDL cholesterol is efficiently delivered directly to the liver and steroidogenic tissues via a selective uptake pathway. SR-BI plays a key role in this process (see text). In some species, including humans but not mice or rats, the cholesterol in HDL can also be transferred by the plasma protein CETP to other lipoproteins for further transport and metabolism. (From Reference 14.)

~65% of HDL cholesteryl esters from the plasma pool via the selective uptake mechanism (40, 42, 44, 52). The significance for lipid transport of the pioneering studies that identified the selective-uptake pathway (38, 39, 42, 43, 66, 67, 83) has not been as widely appreciated as that of endocytosis. This may be, in part, because selective uptake did not fit into the paradigm of receptor-mediated endocytosis and because, despite considerable effort to identify and characterize HDL receptors, there was until recently considerable controversy regarding the existence, as well as the functions and characteristics, of HDL receptors.

By analogy with the LDL system, a key missing element in the study of HDL metabolism was a well-defined HDL receptor that could give a molecular and cellular handle on the system. Using either direct-binding or ligand-blotting assays and a variety of preparations from tissues of different species, many laboratories have reported HDL-binding activities (6–9, 37, 38, 84, 85), some of which may be involved in selective lipid uptake (86, 87) and some which were

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pursued because of their potential role in efflux of cellular cholesterol to HDL (9, 84). However, a molecularly well-defined cell surface HDL receptor for selective cholesterol uptake was only recently identified (14, 23, 39). This is the class B, type I scavenger receptor, SR-BI.

IDENTIFICATION AND CHARACTERIZATION OF SR-BI AS AN HDL RECEPTOR

Multiple Classes of Scavenger Receptors

SR-BI was identified in the course of studies designed to discover and characterize scavenger receptors. Scavenger receptors are cell surface membrane proteins that mediate the binding of chemically modified lipoproteins, such as acetylated LDL or oxidized LDL (88, 89). Scavenger receptor activity was first identified in macrophages by Brown, Goldstein, and colleagues (19, 90). These investigators were examining the mechanisms underlying the deposition of LDL cholesterol in macrophages in artery walls during the initial stages of atherosclerotic plaque formation. Their studies and others led to the macrophage scavenger receptor model of atherogenesis (19, 20, 88, 91–94). This model suggests that LDL enters the artery wall and is chemically modified (e.g. by oxidation), perhaps as a consequence of local inflammatory reactions, and the modified LDL binds to scavenger receptors on the surfaces of macrophages. The scavenger receptors then mediate the efficient endocytosis and lysosomal degradation of the modified lipoprotein. Unlike LDL receptors, macrophage scavenger receptor activity is not substantially regulated by intracellular cholesterol pools. Thus, macrophage scavenger receptors can mediate the massive accumulation of cholesterol and cholesteryl esters and the conversion of macrophages to lipid droplet-filled foam cells. Incubation of cultured macrophage foam cells with acceptors (e.g. HDL or serum) can lead to the efflux of cellular cholesterol to the acceptor (95, 96), and it is commonly thought that HDL-mediated cholesterol efflux from foam cells may play an important part in the cardioprotective effects of HDL. Although the macrophage scavenger receptor model has gained widespread acceptance, much of the supporting evidence is correlative, and further work will be required to determine its validity (see 97 and 98 for recent contributions in this area).

Scavenger Receptor Nomenclature

Analysis of the complex ligand-binding properties of scavenger receptor activities on macrophages and endothelial cells led to the suggestion that there are multiple classes of modified LDL scavenger receptors (99–102). Since the molecular identification of the first macrophage scavenger receptors, SR-AI and SR-AII, in 1990 (103, 104), the cloning of cDNAs encoding a number of distinct scavenger receptors expressed in a wide array of different types of cells has been reported (88, 103–117). It seems likely that additional scavenger receptors remain

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to be identified (118–120). Scavenger receptors with similar structures (usually defined by sequence comparisons) are grouped together to define a "class," identified by a capital letter (class A, class B, etc) (89, 107, 108). Within a class, the individual receptor proteins, or "types," are distinguished by a capital roman numeral. Thus, the first macrophage scavenger receptors to be well-defined molecularly (103, 104) are designated SR-AI and SR-AII for "scavenger receptor, class A, type I" and "scavenger receptor, class A, type II." Different types of scavenger receptors within a given class can arise either because of alternatively splicing RNAs from a single gene [e.g. SR-AI and SR-AII (105, 121) or SR-BI and SR-BII (107, 122)] or because they are the products of different genes (SR-CI and SR-CII) (108; A Pearson and M Krieger, unpublished data). The species from which a scavenger receptor is derived is designated by using lower-case prefix abbreviations [e.g. murine SR-BI (mSR-BI) and human SR-BI (hSR-BI)]. When a previously named and well-characterized protein is found to have scavenger receptor activity, as was the case for CD36 (106, 109), it has been helpful to assign such proteins to a scavenger receptor class (e.g. along with SR-BI, CD36 is a class B scavenger receptor (107); MARCO (110) is a class A scavenger receptor). The structures and functions of several different classes of scavenger receptors have been reviewed elsewhere (88, 89).

Identification of the Class B Scavenger Receptors

CD36 Using a murine macrophage cDNA library and a COS cell expression cloning technique, Endemann and colleagues (109) reported the identification of the first class B scavenger receptor, CD36. The cDNA for CD36 had previously been cloned, and it has been the subject of extensive analysis. CD36 may play a quantitatively significant role in modified LDL binding to macrophages (109). In addition to binding modified LDL, CD36 binds thrombospondin (123), anionic phospholipids (124), rod outer segments (125), long-chain fatty acids (126), collagen (127), and *Plasmodium falciparum*-infected erythrocytes (106). CD36 is expressed in a variety of tissues, including adipose, and in macrophages, epithelial cells, monocytes, endothelial cells, platelets, and a wide variety of cultured lines (112; see 128 for review). CD36 has been reported to be clustered in specialized domains of the plasma membrane called caveolae (see below) (129). Although the physiologic functions of CD36 have not been fully described, it may serve as an adhesion molecule owing to its collagen-binding properties. It has also been proposed to be a long-chain fatty acid transporter (112), a signal transduction molecule (130, 131), and a receptor for the clearance of rod outer segments (125, 132), and CD36 may serve as a receptor on macrophages for damaged or senescent neutrophils (89, 133).

SR-BI Shortly after the identification of CD36 as a scavenger receptor (109), a cDNA for hamster SR-BI (haSR-BI) was isolated by expression cloning (107) from a Chinese hamster ovary cell variant, Var-261, which expresses a scavenger

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receptor activity distinct from that of SR-AI and SR-AII (M Penman, X Huang, S Acton, J Ashkenas, and M Krieger, unpublished data). Unexpectedly, haSR-BI is not responsible for the novel scavenger receptor activity of Var-261 cells (X Huang, S Acton, J Ashkenas, and M Krieger, unpublished data). The murine (mSR-BI) (23), rat (rSR-BI) (134), bovine (bSR-BI) (58) and human (hSR-BI) (111) homologs of haSR-BI have been cloned. hSR-BI, initially identified as a cDNA of unknown function (called CLA-1) (111), has been mapped to human chromosome 12 (12q24.2-qter; 135, 138) and the murine gene maps to the syntenic murine chromosome 5 (136). hSR-BI exhibits tissue-specific expression and in vitro receptor activities similar to those of rodent SR-BI (14, 137, 138; see below). The partial (26) and complete (138) genomic structures for the murine and human SR-BI homologues have been reported.

The principle focus of this review is the role of SR-BI as an HDL receptor. However, SR-BI is a multiligand receptor (88) whose binding specificities differ from those of class A and class C scavenger receptors (23, 24, 88, 89, 107, 139). Thus, SR-BI may have important functions in addition to its HDL receptor activity. SR-BI binds maleylated serum albumin (107) and, along with CD36, was the first anionic phospholipid receptor to be identified (124). This suggested that SR-BI might be involved in recognizing senescent or apoptotic cells (88, 124), and, indeed, such recognition has been observed in vitro (137, 139a, 140), but its physiologic significance, along with that of SR-BI's binding chemically modified LDL (107), is uncertain. Perhaps most relevant to fully understanding normal lipoprotein metabolism, SR-BI can tightly bind native LDL (107, 137), and mediate the uptake of cholesterol from LDL (140a, also see 140b, 256b), although LDL does not effectively compete for HDL binding (23), and SR-BI is much less efficient in mediating the internalization of LDL than is the classic LDL receptor (107; A Rigotti, S Acton, M Krieger, unpublished data). SR-BI can also bind VLDL (141; Acton, Rigotti, and Krieger, unpublished data) Additional work will be required to elucidate the molecular basis and the significance of the broad ligand-binding specificity of SR-BI.

SR-BI Structure and Subcellular Localization

The predicted sequences of the hamster, murine, rat, bovine, and human SR-BI proteins share ~75–80% identity over their 509-amino-acid lengths. The SR-BIs are members of the CD36 superfamily of proteins, which also includes the mammalian proteins CD36 (reviewed in 128) and LIMPII (a lysosomal protein) (142), two *Drosophila melanogaster* proteins, emp (144) and croquemort (a *Drosophila* hemocyte/macrophage receptor) (145), SnmP-1 (a silk moth olfactory neuron membrane protein) (143), and a putative *C. elegans* protein (GenBank Z54270). SR-BI (509 amino acids) and CD36 (472 amino acids) are class B scavenger receptors (both bind modified lipoproteins) (107, 109, 146). Members of the CD36 superfamily, which have about 30% sequence identities, have been proposed to have similar membrane topologies (Figure 4; 106, 107, 128, 147; how-

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ever, see 148 for an alternative model of the topology). They appear to comprise a large extracellular loop, which is anchored to the plasma membrane on each side by transmembrane domains adjacent to short cytoplasmic N- and C-terminal domains (see below). Analysis of the topology of mSR-BI with an anti-C-terminus antipeptide antibody (23, 149) supports the cytoplasmic location of its C-terminus; however, additional studies will be required to definitively establish whether its N-terminal domain is cytoplasmic or extracellular (147, 148). [Croquemort differs from the other CD36 members because it does not have a hydrophobic domain near the carboxyl terminus (145) and is thus presumably anchored to the membrane by its N-terminal hydrophobic domain.] SR-BI and CD36 share sequence similarities throughout their entire extracellular loop domains, including conserved cysteines (five of six) and multiple N-linked glycosylation sites; however, the N- and C-terminal cytoplasmic and transmembrane domains have very little sequence similarity. Alternatively spliced mRNAs have been identified for both CD36 (150) and SR-BI (alternative form designated SR-BII)(122, 138, 151).

Murine SR-BI is heavily N-glycosylated, and this accounts for the difference between its mass predicted from the amino acid sequence (~57 kDa) and that observed by immunochemical methods (~82 kDa) (23, 149; see below). mSR-BI is cotranslationally modified on at least 5 of its 10 high-mannose N-linked oligosaccharide chain sites (149). Some, but not all, of these are processed to complex forms during transit through the Golgi apparatus (149). Metabolic labeling with [^{3}H]palmitate and [^{3}H]myristate demonstrated that mSR-BI is fatty acylated, a characteristic shared with CD36 (147) and other proteins that concentrate in specialized cholesterol- and glycolipid-rich plasma membrane microdomains called caveolae (152, 153). Analysis of point mutants (154) established that the major sites of fatty acylation of mSR-BI are Cys462 and Cys470 and these cysteines (see legend to Figure 4), rather than the potential N-myristoylation site at the N-terminus (149), can also serve as sites of myristoylation. OptiPrep density gradient fractionation of plasma membranes established that mSR-BI copurifies with caveolin-1, a constituent of caveolae; and immunofluorescence microscopy demonstrated that, at the level of resolution of the light microscope, mSR-BI colocalizes with caveolin-1 in punctate microdomains across the surface of cells and on the edges of cultured cells. Thus, mSR-BI colocalizes with caveolae (149), and this raises the possibility that the unique properties of these specialized cholesterol- and sphingolipid-rich, cell surface domains (152, 153) may play a critical role in SR-BI function (149). SR-BII, an isoform of SR-BI in which 42 of the ~45 C-terminal amino acids in the C-terminal cytoplasmic domain (Figure 4) of SR-BI are replaced by 40 residues (122) encoded by a distinct exon (138), is also concentrated in caveolae (151). In vivo, SR-BI has been detected on the surfaces of cells (155–157) in structures resembling the intercellular canaliculi that occur along the junctions of the parenchymal cells of the adrenal cortex (155) and the ovarian corpus luteum (157) and on microvilli and microvillar channels (158; see below).

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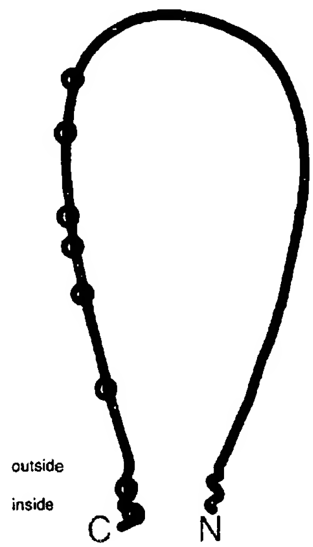

Figure 4 Model of the topology of murine SR-BI. SR-BI, a member of the CD36 superfamily of proteins, is a 509-residue glycoprotein comprising a large extracellular loop (~403 amino acids), which is apparently anchored to the plasma membrane on each side by transmembrane domains (~28 and ~25 resides) which are adjacent to short cytoplasmic N-terminal (~8 residues) and C-terminal (~45 residues) domains. The approximate locations of the cysteines are shown (*circles*). The protein is heavily N-glycosylated, and it is palmitoylated on the cysteines in the C-terminal cytoplasmic and transmembrane domains, Cys^{462} and Cys^{470}.

SR-BI–Mediated HDL Binding

In the course of the analysis of SR-BI's ligand-binding activity in transfected cultured cells expressing SR-BI, my colleagues and I unexpectedly discovered that SR-BI could mediate the high affinity (K_d ~20–30 μg of protein/ml) and saturable binding of radiolabeled human HDL to cells in an EDTA- and apoE-independent fashion (23). Examination of the binding to COS cells expressing mSR-BI of Alexa-HDL, in which the apolipoproteins on the surface of the HDL particles are covalently modified with the green-fluorescing dye Alexa (154), established that HDL binds to the surfaces of the cells in the punctate pattern expected for binding to a receptor clustered in caveolae (see above). Studies with apoA-I, apoA-II, and apoC-III in both lipid-free and phospholipid/cholesterol-complexed forms indicate that each of these HDL apolipoproteins can mediate binding to SR-BI (139). Taken together with the finding that SR-BI can bind apolipoprotein B containing lipoproteins (107, 141), it seems likely that SR-BI is responsible for some of the multilipoprotein- and multiapolipoprotein-binding activities previously described in a variety of cell types and tissues (reviewed in 6–9, 37, 38, 86, 87). It is noteworthy that HDL competes effectively for the

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binding of native LDL to SR-BI, whereas native LDL only poorly blocks the binding of HDL (23). Because of this nonreciprocal cross-competition (also see 159, 160), LDL is not expected to significantly interfere with SR-BI's binding of HDL in vivo. Future studies should help identify the complementary binding sites on SR-BI and its lipoprotein and apolipoprotein ligands.

SR-BI–Mediated Selective Lipid Uptake

Although SR-BI mediates the high-affinity binding of HDL to cells, it does not mediate subsequent endocytosis and lysosomal degradation of the intact particle. Studies with transfected cultured cells, using HDL-containing radioactive ([^{3}H]cholesteryl ester or ether) or fluorescent (DiI) lipids established that SR-BI mediates very efficient cellular selective uptake of HDL lipids (23; Figure 2*b*). Several investigators have shown that selective cholesteryl ester uptake by cells and tissues is not dependent on a single class of lipoprotein or apolipoprotein (38, 39, 42–48, 66–76, 86, 87, 161–163). It seems likely that SR-BI, which is a multilipoprotein and apolipoprotein receptor (see above), may be responsible for some of the previously reported broad lipoprotein-binding specificity of selective lipid transfer. However, it is also possible that there may be more than one cell surface HDL receptor (see below) and that they may have distinct structures, tissue distributions, and functions.

Although the precise molecular mechanism of SR-BI–mediated transfer of lipid from HDL to cells has not yet been described, some features of the mechanism have been uncovered. Before the discovery of SR-BI, selective cholesterol uptake from HDL to cells and tissues was shown to proceed via initial transfer of lipid from HDL to the plasma membrane (77, 78, 164). SR-BI–mediated selective uptake appears to involve a similar mechanism (23, 154). Until recently, it had been uncertain as to whether or not the lipid transfer step of selective uptake was protein facilitated. One possibility, the "docking only" model, was that selective uptake merely required bringing HDL in close proximity to the plasma membrane, and the lipid transfer step would then proceed without mechanistic intervention by the receptor (165). Alternatively, SR-BI might directly and specifically facilitate the lipid transfer step in addition to mediating HDL binding to the cell. This issue has been resolved by comparing the activities of transfected cultured cells expressing either CD36 or SR-BI. hCD36 has been shown to be similar to SR-BI not only in its sequence and subcellular localization (see above), but also in that it can bind HDL with high affinity (141, 154) in a punctate pattern on the surfaces of cells, which resembles the distribution of SR-BI–bound HDL (154, 154a). However, CD36 cannot mediate efficient cellular HDL lipid uptake (15) as does SR-BI. Thus, high-affinity binding to a caveolae-associated cell surface receptor is not sufficient to insure efficient cellular lipid uptake from HDL. Rather, SR-BI–mediated binding combined with SR-BI–dependent facilitated transfer of lipid from the bound HDL particle to the cell appears to be the most likely mechanism for the bulk of the cellular selective uptake of cholesteryl esters from HDL

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observed in vitro and in vivo (see below). Analysis of point mutants and mSR-BI/hCD36 chimeras (154, 154a) suggests that the distinctive ability of mSR-BI to mediate selective lipid uptake is primarily a consequence of its extracellular loop and not of fatty acylation or specific sequences in its two sets of transmembrane and cytoplasmic domains (Figure 4). Interestingly, analysis of the selective uptake activity of SR-BII, an isoform of SR-BI with a divergent C-terminal cytoplasmic domain (see above), indicates that it is ~25% as efficient as SR-BI in mediating selective uptake (151). Thus, in some circumstances the C-terminal cytoplasmic domain may influence the efficiency of selective uptake. It remains to be determined whether the distinctive properties of the specialized cell surface structures in which SR-BI clusters, such as microvillar channels and caveolae, significantly contribute to the mechanism of SR-BI–mediated selective lipid uptake.

SR-BI and Cellular Cholesterol Efflux

HDL-mediated efflux of cholesterol from cells is thought to play an important role in reverse cholesterol transport (Figure 2*b*) and in mediating the cardioprotective effects of HDL (9). Considerable efforts have gone into the investigation of the biochemical and biophysical nature of cellular cholesterol efflux and its physiological relevance (7–9, 36–38). Indeed, much of the effort devoted to identifying and characterizing potential HDL receptors has been focused on receptors which might mediate efflux (e.g. 9, 36–38, 84). Because of microscopic reversibility and observations in mice overexpressing SR-BI, the potential of SR-BI for mediating cholesterol efflux from cells was proposed (24, 25). This proposal was confirmed by experiments in cultured cells (168, 169 140a), which showed that expression of SR-BI in cells can enhance cholesterol efflux, that extracellular phospholipid can influence this efflux, and that the levels of SR-BI expression in a variety of untransfected cultured cells correlate with HDL- and phospholipid-mediated efflux rates. Although high levels of SR-BI expression are seen in a few specific tissues (e.g. steroidogenic cells; see below), low levels of SR-BI can be detected in many tissues and cultured cell types, including fibroblastic cells and macrophages (107, 111, 168). The physiological significance of SR-BI–mediated cholesterol efflux has not been established, but it may play a role in reverse cholesterol transport (Figure 3) and atherogenesis.

PHYSIOLOGICAL RELEVANCE OF SR-BI'S HDL RECEPTOR ACTIVITY

Three lines of evidence, primarily involving in vivo studies, support the original proposal (23) that SR-BI is a physiologically relevant HDL receptor. They are (*a*) description of the tissue distribution of SR-BI expression, (*b*) hormonal regulation of SR-BI expression in vivo and in vitro, and (*c*) alteration of SR-BI expression by immunochemical or genetic techniques.

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Sites of SR-BI Expression In Vivo

Expression in Adults The initial studies of the tissue distribution of SR-BI expression were performed by immunoblot analysis of murine (23) and then rat (156) specimens. SR-BI is most highly expressed in those tissues that were previously shown to be the principal sites of selective lipid uptake from HDL in vivo: the liver, the adrenal gland, and the ovary (23, 156). In addition, low levels of SR-BI were detected in the unstimulated testis and intestine, and significant levels were seen in the mammary glands of pregnant rats (156). The tissue-specific pattern of expression of SR-BI in adult cattle (58) and humans (137, 138) is similar to that observed in rodents—preferential expression in liver and steroidogenic tissue.

The expression of SR-BI in the adrenal gland raises the possibility that SR-BI may play a role in the processing of bacterial lipopolysaccharides (LPS, endotoxin) during gram-negative sepsis. HDL binds endotoxin and can protect against endotoxemia (170–173). In rats, monkeys, and rabbits, the adrenal gland and the liver are principal sites of the accumulation of HDL-bound LPS (174–176). There appears to be coordinate regulation of LPS accumulation, HDL binding, and SR-BI expression in the adrenal gland (155, 156, 174, 175, 177; see below). These findings raise the possibility that SR-BI may play a role in determining the tissue distribution of LPS and may contribute to the acute adrenocortical insufficiency or hemorrhage observed in severe gram-negative sepsis (39, 178–180).

Although substantial amounts of SR-BI mRNA were observed in murine adipose tissue samples and in 3T3-L1 preadipocytes after they had been induced to differentiate into adipocytes in culture (107), very little SR-BI protein was seen in these adipose cells and tissues (23, 156). Thus, although in many cases the relative in vivo levels of SR-BI protein and mRNA in different tissues are correlated (156), the determination of the absolute levels and changes in SR-BI mRNA levels may not always reflect the levels of SR-BI protein and SR-BI activity in tissues or cells.

Immunofluorescence and immunohistochemical studies have shown that SR-BI is expressed primarily on the surfaces of hepatocytes and steroidogenic parenchymal cells such as the zona fasciculata and zona reticularis cells of the adrenal cortex, the thecal cells of unstimulated ovaries, ovarian corpus luteal cells, and the Leydig cells of the testis (155, 156). In the murine adrenal gland, SR-BI was found in numerous circular and oval structures on the adrenocortical-cell surfaces, which appear to represent cross sections through previously described microvilli-rich intercellular channels (155). HDL has been shown to accumulate in these channels (166, 167, 181) and they may play an important role in the selective lipid uptake process (155, 166, 167, 181).

It is noteworthy that hepatic lipase is found preferentially in those adult tissues that express high levels of SR-BI (182). Hepatic lipase can substantially affect the metabolism of HDL and other lipoproteins (15, 80, 183–192). In some cases the catalytic activity of hepatic lipase may be required for its effects on lipoprotein

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metabolism (189), although in others its activity appears to be less important (80, 189). Hepatic lipase stimulates the delivery of cholesterol from HDL to hepatoma cells (80, 190) and isolated perfused liver (191) and increases selective cholesteryl ester uptake from HDL when expressed as a cell surface-anchored form in transfected CHO cells in culture (192; also see below). For these reasons and also because of observed alterations in SR-BI mRNA expression in hepatic lipase-deficient mice (193; see below), it was proposed that hepatic lipase and SR-BI may be acting in concert during selective HDL cholesteryl ester uptake (24, 192, 192a, 192b, 193). Hepatic lipase may remove excess phospholipid from the surfaces of cholesteryl ester-depleted particles, or the core lipids from hepatic lipase-digested particles might be more readily transferred to cells via an SR-BI–mediated mechanism. Examination of the effects of anti-hepatic lipase antibodies on SR-BI and selective uptake in rats in vivo indicate that expression of SR-BI can be altered by lowering the activity of hepatic lipase, but that this activity is not required for SR-BI–mediated uptake of HDL-cholesteryl ester by rat adrenal glands (193a). This is consistent with the initial observation that SR-BI–mediated selective uptake can be observed in transfected CHO cells expressing foreign SR-BI without the addition of exogenous hepatic lipase (23).

Temporal and Spatial Pattern of Expression During Murine Embryogenesis Maternal lipoproteins and endogenous synthesis are sources of the substantial amounts of cholesterol required for membrane synthesis (tissue growth) in the developing embryo and for steroid hormone production in the extraembryonic tissues (194–215). Although the maternal-fetal transport system for lipoprotein cholesterol remains poorly defined, it is generally thought that the placenta and yolk sac play major roles in this process, serving as functionally active interfaces between maternal circulation and the embryo (202, 207–214, 216–220). Initial attempts to explore the possible role of SR-BI in delivering lipid to the developing embryo and extraembryonic tissues have involved immunofluorescence microscopy to define the temporal and spatial pattern of SR-BI expression during rodent embryogenesis (214, 215).

On day 7.5 of mouse embryonic development (E7.5), when gastrulation is complete and formation of the cardiovascular and hematopoietic systems has begun, there is no embryonic circulation or placenta. Within the endometrium-derived decidua, which surrounds the developing embryo and extraembryonic tissues, there are newly formed maternal blood vessels that provide nutrients and remove waste products. At this stage, there is significant SR-BI expression in endothelial cells of the decidua, but little in intraembryonic and extraembryonic tissues. On day E8.5, blood islands expand; vascular structures, including the endocardial tubes, dorsal aorta, and heart develop; somitogenesis occurs; and the developing heart myocardium begins to contract in the absence of embryonic circulation. At this time there is a dramatic increase in SR-BI expression in the trophoblast cells that surround the developing embryo. On day E10, the embryo itself has grown sufficiently large that direct diffusion of nutrients and gases

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apparently cannot support further growth and the placenta becomes a key site for transport of nutrients and for communication between the mother and the fetus (195). At this stage, SR-BI is expressed in both the placenta and yolk sac. The expression in these extraembryonic tissues (e.g. yolk sac visceral endoderm) is correlated with significant uptake of the lipophilic fluorescent dye DiI from DiI-labeled HDL injected into pregnant mice (214). At each stage, SR-BI is expressed on the apical sides of cells, which face the material circulation and are sites that would permit direct interaction with maternal lipoproteins.

Independent analysis of SR-BI expression during hamster embryonic development (215) also showed very high expression in the yolk sac (days E10.5 and E14.5) and significantly less expression in the placenta. Curiously, although SR-BI and apoA-I colocalize on the hamster's yolk sac apical surface, analysis of HDL clearance indicated that very high levels of the entire HDL particle (both apoA-I and cholesterol) are removed from the maternal circulation by the yolk sac. This suggests that, in the hamster, selective uptake alone may not explain the high rate of yolk sac clearance of maternal HDL (215).

Within the murine embryo proper, immunostaining of SR-BI can be seen in the fetal adrenal gland (by day E14.5) and in the hindgut (day E17) outlining the folds of the gut epithelium (214). High levels of SR-BI expression have also been detected in the human fetal adrenal gland (138, 156). Immunofluorescence methods did not detect SR-BI expression in the murine embryonic liver up to day E17.5; however, SR-BI was readily observed in neonatal livers. Although additional studies will be required to fully describe the mechanisms of transport, the studies to date (214, 215) suggest that SR-BI may be involved in the rodent maternal-fetal lipid transport system, helping to provide HDL cholesterol for either membrane or steroid hormone synthesis or both.

Regulation of SR-BI Expression In Vivo

If SR-BI plays a role in selective HDL cholesterol uptake in vivo (23), one would predict that the levels of SR-BI protein would be regulated by physiologic and pharmacologic stimuli that alter sterol metabolism in the tissues expressing this receptor. This has indeed been found to be the case in diverse studies from several laboratories. In vivo and in vitro regulation of SR-BI's expression has been examined in the adrenal gland, ovary, and testis, where SR-BI expression is coordinately regulated with steroidogenesis, and in the liver.

Major Steroidogenic Organs *Adrenal gland.* The steroidogenic cells of the adrenal gland reside in the cortex, which surrounds the nonsteroidogenic medulla, and ACTH is the major endogenous stimulator of adrenocortical glucocorticoid synthesis. A combination of hypolipoproteinemia and ACTH induces a dramatic elevation in adrenal ^{125}I-HDL uptake in rodents (221), and ACTH stimulates adrenal-selective cholesterol uptake from HDL (40, 47, 68, 177, 221). Selective uptake of cholesteryl esters from HDL is thought to take place in HDL-filled

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microvillar channels formed by juxtaposition of microvilli on adrenocortical cells (166). Microvilli and microvillar channels are found in the subendothelial space facing the sinusoid (166) and in intercellular canaliculi along the junctions of adjacent cortical cells (222, 223). In the rat, HDL is found on canalicular microvilli and subendothelial microvilli (222). Consistent with the proposal that SR-BI plays a key role in delivering HDL cholesterol to steroidogenic cells, its distribution on the surfaces of murine adrenocortical cells in vivo is almost identical to that of bound HDL in circular and oval structures that appear to represent intercellular canaliculi (155). Especially high levels of SR-BI expression have been reported in the human fetal adrenal gland (138, 156). The two initial in vivo studies of the regulation of adrenocortical SR-BI expression by tropic hormones involved treating mice with ACTH (155) or rats with very high doses of estrogen (156).

Rigotti et al (155) showed, using both immunoblot and immunohistochemical methods, that ACTH treatment of mice dramatically increases SR-BI protein expression in adrenocortical cells in vivo. It seems likely that ACTH acts directly on the adrenocortical cells, rather than indirectly, because it also stimulates mSR-BI protein expression (155) as well as mRNA (193, 224) expression in cultured murine or human adrenal cells. ACTH induces synthesis of the second messenger cAMP in target cells, and ACTH and dibutyryl cAMP treatments increase SR-BI mRNA levels in primary cultures of human adrenocortical cells coordinately with those of proteins involved in steroidogenesis [e.g. steroidogenic acute regulatory protein (StAR) and cholesterol side-chain cleavage enzyme (P-450scc) (224)]. Because of the reduced serum ACTH levels in patients with Cushing's syndrome, the lower levels of SR-BI mRNA in the normal adrenal tissue adjacent to Cushing's adenoma in these patients are consistent with a role for ACTH in the regulation of SR-BI in humans (224). Furthermore, in vivo administration to mice of the glucocorticoid dexamethasone, which suppresses ACTH secretion and thus adrenocortical steroidogenesis, dramatically suppresses adrenocortical SR-BI protein expression (155). This suggests that the relatively high basal level of SR-BI in this tissue may be caused by endogenous basal ACTH production (155). There are reports of altered adrenal SR-BI mRNA levels in mice with homozygous mutations in the apoA-I (193), hepatic lipase (female only) (193), and lecithin:cholesterol acyltransferase (225) genes, suggesting that the activities of these genes may directly or indirectly affect the expression of SR-BI in the adrenal gland. However, an independent analysis comparing apoA-I knockout to wild-type mice found no alteration in the mutant in SR-BI protein or mRNA levels in the adrenal gland or any of the other organs examined (226). The basis for this discrepancy has not yet been established.

High-dose estrogen treatment can have profound effects on a number of organ systems in some mammals. For example, plasma lipoprotein levels in rats, rabbits, and humans are estrogen sensitive, whereas the levels in mice are relatively estrogen resistant (227–233). In rats, high-dose estrogen treatment dramatically lowers the plasma LDL and HDL levels (227, 228), apparently owing to a substantial increase in hepatic LDL receptor activity (229, 230) and the consequent rapid

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clearance of plasma LDL and apoE-containing HDL (230, 231). Estrogen also stimulates increases in adrenal selective uptake of HDL-cholesteryl esters in rats (234); thus, the first studies of estrogen's effects on SR-BI were conducted in rats.

Immunoblot and immunofluorescence analysis of rat adrenal glands shows that high-dose (supraphysiologic) estrogen treatment (17-ethinyl estradiol, 5 days) increases SR-BI protein expression on the surfaces of cells in the adrenal cortex (156). This increase is accompanied by increases both in apoA-I binding at the same sites, despite dramatically reduced levels of serum lipoproteins, and in adrenocortical uptake of DiI from DiI-HDL injected into the animals (156). Because SR-BI can bind LDL as well as HDL (23, 107), it is possible that SR-BI contributes to estrogen-enhanced adrenal metabolism of LDL in rats.

The mechanism(s) of high-dose estrogen stimulation of adrenocortical SR-BI expression and selective uptake in rats is uncertain; however, it seems likely that tropic hormones are important in mediating these estrogen effects. They may be an indirect consequence of estrogen-induced increases in ACTH production because of suppression of feedback regulation (235, 236) or because of the stress associated with chronic high-dose estrogen administration, or both (155). Consistent with this proposal is the finding that hypophysectomized rats express substantially reduced levels of SR-BI in the adrenal gland (156; K Wyne & HH Hobbs, unpublished observations). As noted above, the effects of estrogen can be species dependent—no significant estrogen-induced changes in SR-BI expression have been observed in murine adrenal glands or in other murine tissues that have been examined (liver, ovary, and testis; see below) (156).

In rodent adrenal glands, the coordinate regulation of SR-BI expression with stress and steroidogenesis is consistent with the proposal that SR-BI mediates delivery of HDL cholesterol for storage and as substrate for hormone synthesis. In humans the function of SR-BI in adrenal glands (137, 138, 156) remains uncertain. It seems likely that LDL is important in delivering cholesterol to the adrenal gland via LDL receptors (e.g. see 200 and 237). However, normal LDL uptake via LDL receptors is not required for essentially normal adrenal function. Plasma cortisol responses to ACTH in homozygous familial hypercholesterolemia patients (LDL receptor deficient) are normal except under conditions of maximal stimulation (238), and individuals with little apoB100 (abetalipoproteinemics) also exhibit no adrenal insufficiency without prolonged exposure to ACTH (239). Thus, it is possible that SR-BI can also supply substrate cholesterol for steroidogenesis in human adrenals, either in normal circumstances or when the LDL receptor pathway cannot provide adequate cholesterol.

Ovary. In the ovary, the two major types of cells that convert cholesterol to steroid hormones are the thecal cells and cells of the corpus luteum. Theca internal cells surrounding developing follicles are stimulated by luteinizing hormone [LH; also human chorionic gonadotropin (hCG)] to produce aromatizable androgens. These steroids are passed on to the granulosa cells for follicle stimulating hormone-inducible conversion to estrogens. After ovulation, granulosa cells differ-

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entiate (luteinize) into luteal cells (corpus luteum) which can produce steroid hormones (e.g. progesterone) directly from cholesterol. The ovary uses HDL cholesterol for steroidogenesis (40, 42, 44, 52–54) and treatment with hCG can induce luteinization and increase cell surface HDL binding sites in the rat corpus luteum (240). HDL binding to ovarian cells is directly correlated with steroidogenic activity (53). In the unstimulated ovaries of mature rats, SR-BI protein (156) and mRNA (134) expression are seen in thecal cells—consistent with their role in steroidogenesis in the unstimulated ovary (40)—and in the granulosa cells of the corpus luteum. In cows, SR-BI mRNA is also readily detected in the corpus luteum (58). The corpus luteal cells, just as the adrenocortical cells (see above), have a system of microvilli on their cell surfaces which form lipoprotein-filled channels proposed to play a key role in selective lipid uptake (72, 167). The surface expression of SR-BI on these cells in rats (156) has recently been shown to include the presence of SR-BI in these specialized structures (157, 158). In unstimulated, immature (21-day-old) rat ovaries, expression of SR-BI mRNA in thecal cells is low (241, 242). Potential functions of SR-BI have been explored by determining the effects of estrogen and gonadotropins on changes in ovarian SR-BI expression in vivo and in vitro.

Estrogen administration to rats induces increases in (*a*) ovarian weight, (*b*) the size and number of follicles, (*c*) development of the progesterone-producing corpus luteal cells (156), and (*d*) ovarian selective uptake of HDL cholesteryl ester (234, 243). In hypophysectomized rats it also has been reported to increase 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity and the depletion of the cholesteryl ester content of these cells, presumably owing to enhanced steroidogenesis (244).

In rats, high-dose estrogen treatment dramatically alters the cellular distribution of SR-BI protein expression in the ovary (156). It induces an apparent decrease in thecal SR-BI protein expression and a substantial increase in corpus luteal expression (156). The estrogen-induced increases in SR-BI expression in the ovary and adrenal gland (155, see above) in vivo are accompanied by increased steroidogenic cell uptake of the fluorescent lipid DiI from DiI-HDL (156). [Several investigators have shown that DiI transfer from DiI-labeled HDL to cells provides a reliable measure of the initial steps of selective cholesteryl ester uptake (23, 245).] However, caution must be exercised in interpreting the results of experiments comparing clearance and tissue uptake when the endogenous lipoprotein pools differ significantly in the different experimental groups (246). Such cases include the dramatically lower HDL in estrogen-treated rats relative to control animals (156) or mice with altered hepatic SR-BI expression (25, 26, 247).

Treatment of immature female rats with either pregnant-mare serum gonadotropin (PMSG) or hCG, both inducers of follicular growth, induces a strong and rapid increase in SR-BI mRNA expression in the theca interna cells, presumably via the LH/hCG receptor (241, 242). This induction is accompanied by induction of steroidogenic enzymes and StAR (steroidogenic acute regulatory protein) in this tissue (241, 244) and thus parallels the observations in adrenocor-

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tical (see above) and testicular Leydig (156; see below) cells. An in vivo pulse of hCG (luteinizing pulse) given subsequent to the induction of folliculogenesis by initial PMSG treatment induces both the formation of corpus luteum and the expression of SR-BI mRNA in the luteal cells (242). In both rats and cows, increases in SR-BI mRNA expression are observed as the corpus luteum matures (58, 134, 242). In experiments in cows in which the effects of natural luteinization during the estrus cycle, rather than hormone treatments, were examined, SR-BI mRNA levels were reported to increase sevenfold as granulosa cells matured into corpus luteum over a 7-day period after ovulation (58). This increase appears to result from the direct effects of tropic hormones on the granulosa cells, because in vitro luteinization of cultures of bovine (58) or rat (158, 241) granulosa cells by either tropic hormones, inducers of adenylate cyclase, or dibutyryl cAMP induces coordinate increases in SR-BI mRNA and progestin output. In the rat cells, this increase has been shown to correlate with increased selective cholesterol uptake from HDL (158, 243). Additional in vivo correlation of selective uptake and SR-BI expression has been observed in luteinized and hormone-desensitized rat ovarian models (157).

Testis. HDL-cholesterol is the preferred source of substrate for steroid synthesis in the rat testis (52). Testicular HDL-binding and cholesteryl ester uptake (248–250) is stimulated by hCG treatment, although, unlike estrogen (see above), hCG administration has little effect on the plasma levels of cholesterol (40, 248). The characteristics of HDL binding to testicular membranes (251) are similar to those for binding to SR-BI (23, 24). Administration of hCG to male rats dramatically (>20-fold) induces in vivo SR-BI protein expression, exclusively in the steroidogenic testicular Leydig cells (156). This effect is accompanied by increased in vivo fluorescent lipid uptake from DiI-HDL (156).

Taken together, experiments examining the adrenal gland, ovary, and testis all provide very strong correlative support for the hypothesis that, in steroidogenic tissues, SR-BI mediates selective uptake of cholesteryl esters to supply substrate for steroid hormone synthesis.

Mammary gland Mammary gland cells from pregnant rats express higher levels of SR-BI than those from nonpregnant controls (156). This observation suggests an additional tissue in which SR-BI expression may be under hormonal control, although the function of SR-BI in pregnant mammary gland (e.g. delivery of lipid to growing tissue or forming milk) and the mechanism underlying its regulation have not been determined.

Liver Although the specific activity of SR-BI expression in the liver is not as great as in some steroidogenic tissues, the large size of the liver makes it the major site of SR-BI expression as well as selective uptake of HDL cholesteryl esters (23, 24, 42, 44, 156). The effects of both high-dose estrogen administration (which induces high levels of hepatic LDL receptor expression in rats) and

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cholesterol feeding on the levels of expression of hepatic SR-BI in rats in vivo have been described (252, 252a). In addition, the effects of pregnancy and lactation on hepatic SR-BI expression in rats has recently been reported (253a).

Estrogen regulation in vivo Administration of estrogen to rats has been shown to reduce hepatic selective uptake of HDL cholesteryl esters (234). In rats, high-dose estrogen treatment dramatically suppresses in vivo hepatic SR-BI protein expression (156). Subsequent analysis confirmed and extended this work by showing that estrogen treatment suppress SR-BI expression in parenchymal cells (hepatocytes) in vivo and that there is a coordinate reduction in hepatocyte selective uptake of HDL cholesteryl esters in vivo and in vitro (252, 252a). Unexpectedly, the same estrogen treatment induces increases in both SR-BI expression and selective uptake in the far less abundant hepatic Kupffer cells (sinusoidal macrophages) (252). These correlations of hepatic SR-BI expression and selective uptake (156, 234, 252) in vivo, along with in vitro HDL binding/competition studies that showed that SR-BI ligands can inhibit selective uptake into hepatocytes (253, 253b), support the proposal that SR-BI is an important mediator of hepatic selective uptake (23).

In humans, estrogen induces reduced levels of plasma LDL-cholesterol and increased plasma HDL-cholesterol (254) and clearly plays a role in the relatively reduced cardiac risk of premenopausal women (1, 255). The mechanisms underlying the higher plasma HDL-cholesterol levels in estrogen-treated postmenopausal and premenopausal women are not yet clear. Increased hepatic synthesis of apoA-I in estrogen-treated women may be involved (256). As indicated above, there are species-dependent differences in the effects of estrogen on lipoprotein metabolism and SR-BI expression [no substantial estrogen-induced changes in hepatic SR-BI in the mouse (156)]. Thus, caution must be exercised when extrapolating from the rat to the human. Nevertheless, it is intriguing to speculate that estrogen treatment may suppress hepatic SR-BI expression in humans and as a consequence directly contribute to higher HDL-cholesterol levels via reduced selective uptake (25, 26, 156). Additional experiments will be required to explore the connections in humans between estrogen, hepatic SR-BI, plasma HDL, and atherosclerosis (see below).

Dietary cholesterol regulation A 2-week high-cholesterol diet was found to simultaneously suppress in rat parenchymal cells and induce in the corresponding Kupffer cells both SR-BI expression and selective uptake (252, 252a). These results provide the most convincing evidence to date for a role of cholesterol in regulating SR-BI expression, although the mechanism remains uncertain.

Regulation during pregnancy and lactation In rats hepatic levels of SR-BI have been reported to increase about twofold in late pregnancy and at birth, return to nonpregnant control levels by three days postpartum, drop to below control values late (day 19) in lactation, and then return to normal in the post-lactating female (253a). A striking result in this study was that the regulation of hepatic

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LDL receptors and SR-BI were significantly different, suggesting that their functions during late pregnancy and lactation may differ significantly (253a).

Mechanisms Underlying SR-BI Regulation Currently, relatively little is known about the molecular mechanisms underlying the regulation of SR-BI expression (58, 134, 155, 156, 193, 224, 225, 241, 242). As outlined above, we know that tropic hormones, such as ACTH and hCG, and the second messenger they induce, cAMP, can directly induce SR-BI protein and RNA expression in responsive cultured cells and/or tissues in vivo. We also know that high-dose estrogen treatment can induce or suppress SR-BI expression in vivo; however, this estrogen responsiveness is species dependent (rats are sensitive to estrogen, but mice are not) and may be due to indirect effects, rather than or in addition to direct effects, of estrogen on the cells whose SR-BI levels are altered. The observations that in certain cases in vivo hormonal or dietary treatments can induce SR-BI expression in some tissues or cell types and suppress it in others clearly indicate that there are cell type-dependent differences in the systems that regulate SR-BI expression (156, 252). There has been speculation that SR-BI expression could be sensitive to cellular cholesterol content (156, 157, 193, 225, 252), with some of the most persuasive evidence coming from cholesterol feeding studies (252). In studies designed to alter the cholesterol-regulatory pools in ACTH-sensitive Y1-BS1 murine adrenal cells in culture, we have treated the cells with either cholesterol plus 25-hydroxycholesterol or an HMG CoA reductase inhibitor and have not detected changes in SR-BI protein expression (A Rigotti, X Huang, J Babbit & M Krieger, unpublished data). This may be caused by peculiarities of this cell line, or the nature of adrenocortical cells in general. It is possible that in some types of cells or tissues there is either cholesterol-independent hormonal regulation, hormone-independent regulation by cholesterol, or regulation by both cholesterol content and hormonal signaling.

The best initial insights into the molecular basis of this regulation have come from the identification of consensus binding-site sequences for several transcription factors in the putative promoter of the human SR-BI gene (138). These include C/EBP (which is cAMP and gonadotrophin responsive in endocrine cells, as is SR-BI expression), SREBP-1 (a key mediator of cholesterol-regulated gene expression), and SF-1 (an essential activator of the expression of steroidogenic enzymes and StAR). There is considerable similarity, but not identity, in the distribution of SF-1 and SR-BI expression, and studies in cultured adrenocortical cells have shown that SF-1 is likely to play an important role regulating SR-BI expression in adrenocortical cells (138).

Consequences of the Manipulation of SR-BI Expression on HDL Metabolism In Vitro and In Vivo

The in vitro studies of SR-BI activity in transfected cultured cells, the tissue distribution of SR-BI, and the in vivo and in vitro regulation of SR-BI expression

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all suggest that SR-BI is a physiologically important HDL receptor for selective lipid uptake. A series of in vitro and in vivo studies (25–27, 247) have now directly established that this is so.

Blocking Antibodies Temel et al (27) generated antibodies to a portion of the extracellular domain of mSR-BI, which inhibit HDL binding. These antibodies can inhibit selective uptake of HDL cholesteryl esters and conversion of HDL-derived cholesterol to steroid hormones by ACTH-stimulated cultured adrenocortical cells (27). Because earlier studies involved analysis of selective uptake in transfected cells or correlations of SR-BI expression and selective uptake, the blocking antibody work represented the first direct evidence that SR-BI was involved in mediating selective uptake in a physiologic system.

Hepatic Overexpression In the first study to directly manipulate SR-BI levels in vivo, Kozarsky et al (25) used infection of mice with an mSR-BI-encoding adenovirus to transiently overexpress SR-BI on both sinusoidal and canalicular surfaces of murine hepatocytes. This overexpression led to the virtual disappearance of plasma HDL and a doubling of biliary cholesterol 3 days after infection, after which the levels of hepatic SR-BI gradually declined and the levels of HDL slowly returned to normal. The effects of hepatic overexpression of SR-BI on plasma HDL cholesterol and biliary cholesterol have recently been confirmed in transgenic mice which overexpress hepatic SR-BI (256a–c). Analysis of HDL metabolism in the adenovirus-infected SR-BI overexpressing animals was consistent with SR-BI mediating selective lipid uptake (25). There was also a drop in plasma levels of apoA-I, possibly because the increased hepatic selective uptake of HDL cholesteryl esters was accompanied by increased clearance of HDL's apolipoprotein components by the kidney, the tissue that apparently is normally responsible for this clearance (42, 44). The reciprocal relationship between plasma HDL cholesterol and biliary cholesterol levels observed in control and mSR-BI–overexpressing mice is reminiscent of that seen for plasma HDL cholesterol and biliary cholesterol saturation in humans (257). In this regard, it is interesting that high-dose estrogen treatment, which decreases SR-BI expression in rats (156; see above), also suppresses biliary cholesterol secretion in rats (258). These observations coupled with the observation of increased transfer of fluorescent lipid from HDL to the bile in the SR-BI–overexpressing mice (25) suggest that a significant portion of the increase in biliary cholesterol in SR-BI–overexpressing mice was derived from plasma HDL. Thus, SR-BI–mediated biliary cholesterol secretion might influence biliary cholesterol concentrations and potentially influence the likelihood of gallstone formation (25, 259). As noted above, the experiments with the SR-BI–overexpressing mice also provide indirect support for the suggestion, subsequently directly established (168, 169), that SR-BI would have the ability to mediate cholesterol efflux.

Adenovirus-mediated hepatic overexpression of SR-BI has also been used to assess the potential influence of SR-BI on atherogenesis in LDL receptor knock-

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out mice (259a) fed a high-fat/high-cholesterol (western) diet (KF Kozarsky, MH Donahee, JM Glick, M Krieger & DJ Rader, submitted for publication). They showed that injection with the SR-BI expressing adenovirus when compared with injection of control virus decreases plasma HDL cholesterol and non-HDL (includes VLDL, IDL, LDL) cholesterol levels, as might be expected from a receptor that can bind both HDL and several apoB-containing lipoproteins (23, 107, 141). Most important, they found that adenovirus injection causes a striking reduction in atherosclerotic lesion size in as little as 4 weeks after injection. The mean HDL cholesterol levels are significantly correlated with the reduced levels of atherosclerosis (however see 259b). Although additional studies will be required to precisely define the mechanism underlying these observations, an attractive explanation is that hepatic overexpression of SR-BI results in increased net flux of cholesterol from the periphery to the liver and then out of the body, i.e. increased reverse cholesterol transport. Thus, in at least some circumstances, the rate of reverse cholesterol transport may be a better indicator of atherosclerotic risk than total plasma HDL cholesterol levels. It seems clear that the influence of decreasing or increasing HDL cholesterol levels on atherogenesis will depend on the mechanism by which the HDL cholesterol levels are modulated. These findings and recent atherosclerosis studies with SR-BI knockout mice (see below) provide additional support for the suggestion that SR-BI may provide a novel target for intervention in cardiovascular disease (14, 25, 26).

Targeted Gene Inactivation: Knockout Mice Definitive evidence for the physiological role of SR-BI in HDL metabolism came with the first analysis of the properties of mice carrying a targeted disruption (null mutation, no protein product) in the SR-BI gene (26). Standard gene targeting technology was used to delete from embryonic stem cells a short segment of the SR-BI gene. This segment included a 5′ untranslated sequence, the putative N-terminal cytoplasmic domain, a portion of the putative N-terminal transmembrane domain that probably also functions as an uncleaved leader sequence for insertion into the ER during biogenesis, and a portion of the adjacent downstream intron. The heterozygous and homozygous mutant mice, which express either ~50% or no SR-BI protein, respectively, are viable and look normal (weight, general appearance, and behavior), and the males are fertile. However, there is a reduction (non-Mendelian distribution) in the numbers of homozygous offspring relative to wild-type offspring from F1 heterozygous intercrosses. This indicates that there may be partially penetrant effects of the mutation either on neonatal survival or on embryonic development. This finding is consistent with the distribution of SR-BI on the maternal surfaces of cells in the placenta and yolk sac during embryonic development and the proposal that SR-BI may provide nutrients to the developing embryo (214; see above). Without this route for nutrient delivery, embryonic development may be compromised.

Relative to wild-type controls, heterozygous and homozygous SR-BI mutants have substantially increased plasma cholesterol concentrations (30–40% and

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~2.2-fold, respectively) (26). The HDL in the heterozygotes is sometimes slightly larger than that in wild-type animals. Relative to the HDL in wild-type and heterozygous animals, the HDL in the homozygous mutants is substantially larger and more heterogeneous in size and carries a significantly greater amount of apoE. Despite the substantial increases in HDL cholesterol in the SR-BI mutants, there is no statistically significant change in their levels of plasma apoA-I, consistent with SR-BI's function as a receptor for selective uptake. These findings established that the gene encoding SR-BI can play a key role in determining the levels of plasma HDL cholesterol in mice, undoubtedly because reduced expression of SR-BI results in decreased hepatic selective cholesterol uptake. Some of the results and conclusions regarding the effects of SR-BI on plasma HDL metabolism drawn from analysis of the null mutants (26) were subsequently confirmed by analysis, including direct studies of selective uptake, of mice (247) with a partial reduction in SR-BI expression caused by an insertion in a putative promoter region approximately 2 kb upstream of the first exon of the SR-BI gene (138, 247). As expected (23–27), reduced hepatic SR-BI expression resulted in reduced hepatic selective uptake (247).

In addition to its effects on plasma HDL cholesterol, the genetic reduction in SR-BI expression influences cholesterol metabolism in steroidogenic tissues (26). There is a gene dose-dependent decrease in cholesterol content in the SR-BI null mutants in both the adrenal gland (reductions of 42% in heterozygotes and 72% in homozygote mutants) (26) and the ovary (259c). These findings directly established that SR-BI plays a key role in providing cholesterol for accumulation of cholesterol stores in steroidogenic tissue in vivo. The decrease in adrenal cholesterol in SR-BI homozygous mutants resembles the cholesterol depletion observed in other murine homozygous mutants, including lecithin:cholesterol acyltransferase knockouts (225), ACAT knockouts (260), and, as one might expect, mutants in the SR-BI ligand apoA-I (181).

The SR-BI knockout mice are now being used to assess further the influence of SR-BI on HDL metabolism and its role in cardiovascular and endocrine/reproductive pathophysiology. The most striking endocrine/reproductive disruption observed in homozygous null SR-BI knockout mice is that the females, but not the males, appear to be infertile, whereas the heterozygous null females are fertile (259c). The mechanisms responsible for the infertility have not yet been identified. It would not be surprising if abnormal steroidogenesis due to inadequate delivery of HDL cholesterol played an important role.

To examine the effects of disrupting the SR-BI gene on atherogenesis, SR-BI knockout mice were crossed with apoE knockout mice (259c, 262, 263). There was a significant increase in plasma cholesterol (primarily in lipoproteins that size fractionate with VLDL and IDL/LDL) in the double knockouts relative to the single knockout apoE and SR-BI mutant control animals. Thus, SR-BI appears to directly or indirectly influence VLDL and ILD/LDL metabolism—a finding consistent with earlier work that demonstrated that apoB-containing lipoproteins can bind to SR-BI (23, 107, 141; S Acton, A Rigotti & M

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Krieger, unpublished data). There was also extensive atherosclerosis in the hearts of the double knockout animals in as little as 5 weeks after birth, whereas there was virtually no lesion development in the control single knockout animals (SR-BI knockout, apoE knockout). This result is in line with the results (see above) for atherosclerosis studies employing adenovirus-mediated hepatic overexpression of SR-BI in the western-diet–fed LDL receptor knockout mouse. Although overexpression of SR-BI led to lower atherosclerosis, elimination of SR-BI expression dramatically increased atherosclerosis in these mouse models. There are several potential mechanisms that could increase the atherogenesis in apoE knockout mice lacking SR-BI activity, including altered lipoprotein structures and potential changes in cholesterol efflux from peripheral cells. (In addition, other abnormal phenotypes of the double knockout mice have been observed and are currently under investigation.) However, when considered in the light of the work done on hepatic overexpression, it seems possible that decreased reverse cholesterol transport in the double knockout mice may play a critical role in the accelerated atherosclerosis.

SUMMARY

The in vitro and in vivo studies, involving cell biology and physiologic, pharmacologic, and genetic analysis of SR-BI structure and function, all provide definitive support for the proposal that, at least in rodents, SR-BI is a high-affinity, cell surface HDL receptor that mediates physiologically relevant selective cholesterol transport and plays a key role in controlling plasma HDL cholesterol concentration and the delivery of cholesterol to steriodogenic tissue. In mice, SR-BI clearly plays a role in reverse cholesterol transport and substantial increases or decreases in SR-BI activity can significantly effect endocrine/reproductive (female fertility) and cardiovascular (atherosclerosis) pathophysiology. Based on the in vitro activity, tissue distribution and regulation of the human SR-BI (137, 138, 141, 224), it is reasonable to suggest that in humans SR-BI may play a similar role in controlling plasma HDL. If it does, SR-BI's activity may influence the risk for and development of atherosclerosis, and SR-BI may be an attractive candidate for prevention of or therapeutic intervention in this disease (14, 25, 26).

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Commentary

The "best" of cholesterols, the "worst" of cholesterols: A tale of two receptors

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Cardiovascular disease is the number one killer in the U.S., and atherosclerosis is the major cause of heart disease and stroke (1). It is widely appreciated that cholesterol plays an important role in atherogenesis. Normally, most cholesterol serves as a structural element in the walls of cells, whereas much of the rest is in transit through the blood or functions as the starting material for the synthesis of bile acids in the liver, steroid hormones in endocrine cells (e.g., adrenal gland, ovary, testes), and vitamin D in skin. The transport of cholesterol and other lipids through the circulatory system is facilitated by their packaging into lipoprotein carriers. These spherical particles comprise protein and phospholipid shells surrounding a core of neutral lipid, including unesterified ("free") or esterified cholesterol and triglycerides. Risk for atherosclerosis increases with increasing concentrations of low density lipoprotein (LDL) cholesterol whereas risk is inversely proportional to the levels of high density lipoprotein (HDL) cholesterol (2, 3). The receptor-mediated control of plasma LDL levels has been well-defined (4, 5), and very recent studies have now provided new insights into HDL metabolism (6–11).

In 1974, Michael Brown, Joseph Goldstein, and colleagues began publishing a classic series of papers that described the receptor-mediated cellular metabolism of LDL (4, 12). Their work defined how the LDL receptor influences LDL metabolism in the body and helps to determine blood LDL levels. Fig. 1 summarizes in a simplified form the role of LDL in cholesterol transport. In brief, the liver synthesizes a precursor lipoprotein (very low density lipoprotein, VLDL) that is converted during circulation to intermediate density lipoprotein (IDL) and then to LDL (13). The majority of the LDL receptors expressed in the body are on the surfaces of liver cells, although virtually all other tissues ("peripheral tissues") express some LDL receptors. LDL receptors, located in specialized indentations in the cell membrane called coated pits, specifically and tightly bind LDL. After binding, the receptor–lipoprotein complex is internalized by the cells via coated pits and vesicles, and the entire LDL particle is delivered to lysosomes, wherein it is disassembled by enzymatic hydrolysis, releasing cholesterol for subsequent cellular metabolism. This whole-particle uptake pathway is called "receptor-mediated endocytosis" (14). Cholesterol-mediated feedback regulation of both the levels of LDL receptors and cellular cholesterol biosynthesis help ensure cellular cholesterol homeostasis. Genetic defects in the LDL receptor in humans result in familial hypercholesterolemia, a disease characterized by elevated plasma LDL cholesterol and premature atherosclerosis and heart attacks (5). One attractive hypothesis for the deleterious effects of excess plasma LDL cholesterol is that the LDL enters the artery wall, is chemically modified, and then is recognized by a special class of receptors, called macrophage scavenger receptors, that mediate the cellular accumulation of the LDL cholesterol in the artery, eventually leading to the formation of an atherosclerotic lesion (15, 16). A major breakthrough in the pharmacologic treatment of hypercholesterolemia has been the development of the "statin" class of 3-hydroxy-3-methylglutaryl-CoA reductase inhibitory drugs (17). 3-Hydroxy-3-methylglutaryl-CoA reductase is the rate controlling enzyme in cholesterol biosynthesis, and its inhibition in the liver stimulates LDL receptor expression. As a consequence, both plasma LDL cholesterol levels and the risk for atherosclerosis decrease. The discovery and analysis of the LDL receptor system has had a profound impact on cell biology, physiology, and medicine.

What about the counterpart of LDL: HDL and its cellular metabolism? HDL has been the subject of intense study for decades and much has been learned, especially about its constituent parts and its dynamic remodeling in the plasma. In rodents, in which HDL transports most of the plasma cholesterol, HDL has been shown to be an important source of cholesterol for biliary excretion and steroidogenesis. About 15 years ago, reports from Pitman and coworkers (18) and subsequently others indicated that there exists a mechanism for the delivery of HDL cholesterol to cells that is fundamentally different from receptor-mediated endocytosis (refs. 19 and 20, reviewed in refs. 21 and 7). This mechanism is called "selective lipid uptake" because, after HDL binds to cells, only some of the components of the HDL particle enters the cells. In particular, HDL cholesterol (primarily in the form of cholesteryl esters) is transferred efficiently and the lipid-depleted HDL particles subsequently dissociate from the cells and re-enter the circulation. *In vivo*, the highest levels of selective uptake are seen in the liver and steroidogenic organs. Fig. 2 indicates how selective uptake of the cholesteryl esters of HDL might be linked to the proposed role of HDL in removing free cholesterol from peripheral tissues (including the arterial wall and potential sites of atherosclerotic lesions). The overall HDL-mediated movement of cholesterol from peripheral tissues to the liver is called "reverse cholesterol transport" (22). In some species (e.g., humans but not mice), the cholesteryl esters in HDL also can be transferred to other lipoproteins for further transport and metabolism (23). The significance of the pioneering studies that identified the selective uptake pathway (7, 18–21) was not initially as widely appreciated as that of endocytosis, perhaps in part because the mechanism did not fit into the paradigm of receptor-mediated endocytosis and because there was considerable controversy regarding the existence, as well as the functions and characteristics, of HDL receptors.

By analogy with the LDL system, a key missing element in the study of HDL metabolism was a well defined HDL receptor, which could give a molecular and cellular handle on the system. Approximately 2 years ago, the class B type I scavenger receptor (SR-BI), was unexpectedly shown to be the first molecularly well characterized HDL receptor (6). When expressed on the surfaces of cultured mammalian cells, this protein bound HDL (apparently via its main protein component, apoA-I, ref. 24) and mediated selective uptake of HDL lipids. Furthermore, SR-BI was found to be expressed in mice (6) [and subsequently in rats (25) and humans (26, 27)] at high levels in precisely those tissues

Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; SR-BI, class B type I scavenger receptor.

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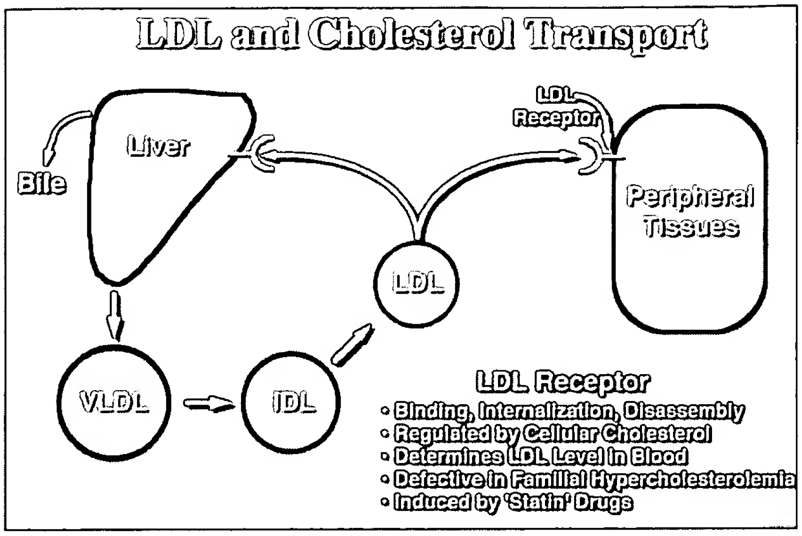

FIG. 1. LDL and the LDL receptor in cholesterol transport. The liver secretes the triglyceride-rich VLDL, which is converted to IDL and then to cholesterol-rich LDL. Plasma LDL cholesterol levels are controlled by the receptor-mediated endocytic clearance of LDL from the circulation by LDL receptors. These receptors are expressed most abundantly in the liver but also are found in many other tissues (see text for details).

that previously had been shown to exhibit the bulk of selective uptake of HDL cholesterol *in vivo* (ref. 18, Fig. 2). The temporal and spatial expression of SR-BI during murine embryogenesis also was consistent with a role of SR-BI in delivering cholesterol to the developing fetus (28). Additional correlative evidence for a role for SR-BI in HDL cholesterol metabolism came from studies of the effects on SR-BI expression of hormones, which induce or suppress steroid hormone synthesis. *In vivo* studies with intact mice and rats, as well as murine and human cultured cell lines, established that SR-BI expression was regulated coordinately with steroidogenesis in the adrenal gland, testes, and ovary (29–33, 47, 48) and that, within these tissues, SR-BI specifically was expressed in the steroidogenic cells (29, 30, 47, 48). Additional *in vitro* evidence suggesting involvement of SR-BI in hepatic selective uptake has appeared (34).

A series of *in vitro* and *in vivo* studies last year (8–10), along with the paper by Varban *et al.* (11) in this issue of the *Proceedings*, have focused on more directly determining whether SR-BI is a physiologically relevant HDL receptor for selective lipid uptake. Temel *et al.* (10) showed that a SR-BI-specific blocking antibody could inhibit selective uptake of HDL cholesteryl esters and conversion of HDL-derived cholesterol to steroid hormones in cultured adrenocortical cells. This finding provided the first evidence that SR-BI was involved directly in mediating selective uptake in a physiologic system. In the first study, which directly manipulated SR-BI levels *in vivo*, Kozarzky *et al.* (8) used an adenovirus to overexpress SR-BI on both sinusoidal and canalicular surfaces of hepatocytes in mice. This resulted in the virtual disappearance of plasma HDL and a doubling of biliary cholesterol. This work strongly suggested that SR-BI may play key roles in hepatic HDL metabolism, in determining plasma HDL concentrations, and possibly in mediating cholesterol efflux from cells. Definitive evidence for the physiological role of SR-BI in HDL metabolism came with the report by Rigotti *et al.* (9) of the first targeted disruption (null mutation, no protein product) of the SR-BI gene in mice. Relative to wild-type controls, heterozygous and homozygous mutants had substantially increased plasma cholesterol concentrations (30–40% and ≈2.2-fold, respectively). There was a slight increase in HDL size in the heterozygous mutants and a substantial increase in the size of HDL and its heterogeneity in the homozygous mutants. These results established that the gene encoding SR-BI can play a key role in determining the levels of plasma HDL cholesterol in mice, almost certainly because reduced expression of SR-BI resulted in decreased selective cholesterol uptake in the liver. In addition, there was a striking gene dose-dependent decrease in adrenal gland cholesterol content in the null mutants (42 and 72% reductions, respectively), establishing that SR-BI plays a key role in providing cholesterol for accumulation of cholesterol stores in steroidogenic tissue *in vivo*. Based on the *in vitro* activity and tissue distribution of the human SR-BI (26, 27, 44), it is reasonable to suggest that SR-BI may play a similar role in controlling

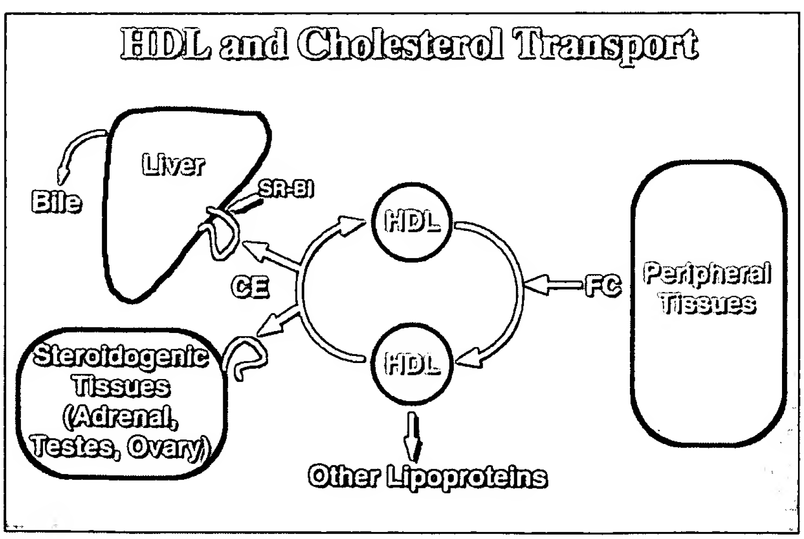

FIG. 2. HDL and the HDL receptor SR-BI in cholesterol transport. HDL is thought to remove unesterified, or "free" cholesterol (FC) from peripheral tissues, after which most of the cholesterol is converted to cholesteryl ester (CE) by enzymes in the plasma. Subsequently, HDL cholesterol is efficiently delivered directly to the liver and steroidogenic tissues via a selective uptake pathway and SR-BI or, in some species, transferred to other lipoproteins for additional transport and metabolism.

plasma HDL in humans. Thus, activity of SR-BI may influence the development and progression of atherosclerosis, and SR-BI is an attractive candidate for therapeutic intervention in this disease (8, 9).

Varban *et al.* (11) now report the second application of gene targeting technology to study SR-BI function. They generated mice with a large (≈13.5 kilobase) insertion in a putative promoter region ≈2-kilobase upstream of the first exon of the SR-BI gene (9, 27). This mutation appears to have no effects on the structure of the SR-BI protein but rather alters the levels of SR-BI expression. A noteworthy consequence of this insertion is that, in homozygous mutants, there was an ≈50% reduction in hepatic SR-BI expression, which was accompanied by a 51% increase in plasma HDL cholesterol. In these and other key properties (FPLC lipoprotein cholesterol profile and slight increase in HDL size), the homozygous insertional mutants remarkably closely resemble the previously described heterozygous null mutants (9). Thus, this study independently confirms that SR-BI can play a key role in determining murine plasma HDL levels. Analysis of plasma lipoproteins in the null mutants (9) in conjunction with the initial *in vitro* studies (6) and the blocking antibody work (10) provided strong indirect evidence that SR-BI mediates selective uptake *in vivo*. An important additional contribution by Varban *et al.* (11) is that they have measured directly selective HDL cholesteryl ester uptake in the insertional mutants and controls. They have obtained compelling additional evidence for the crucial role of SR-BI in hepatic selective uptake *in vivo*.

The mechanism by which the insertion described by Varban *et al.* (11) blunts SR-BI expression remains to be determined. It is of interest that this insertion substantially reduces basal levels of SR-BI expression in the adrenal gland but does not appear to interfere with the stress-mediated induction of adrenal SR-BI expression by ACTH via cAMP (30–32). Although little is known about the molecular mechanisms underlying the regulation of SR-BI expression (29–33, 46–48), Hobbs and colleagues (27) recently have identified consensus-binding site sequences for several transcription factors in the putative promoter of the human SR-BI gene. These include C/EBP (cAMP and gonadotrophin responsive in endocrine cells, as is SR-BI expression), SREBP-1 (mediates cholesterol-regulated gene expression), and SF-1 (activates expression of several genes involved with steroidogenesis). There is striking overlap, but not identity, in the tissue distribution of SF-1 and SR-BI expression. Cao *et al.* (27) showed that SF-1 is likely to play an important role regulating SR-BI expression in adrenocortical cells. Because the binding sequences for these transcription factors lie within the first kilobase upstream of the transcriptional start site of human SR-BI, they are presumably ≈1-kilobase downstream from the insertion in the murine gene generated by Varban *et al.*, and therefore their activities might not be attenuated in the mutant mice.

All of the above cell biological, physiological, pharmacological, immunochemical, and genetic analyses of SR-BI now have provided definitive evidence for the function of SR-BI. It is a high affinity, cell-surface HDL receptor that mediates physiologically relevant selective cholesterol transport, and it plays a key role in controlling plasma HDL cholesterol concentration, HDL structure, and delivery of cholesterol to steriodogenic tissues (7, 9). However, many questions regarding the structure and function of SR-BI remain unanswered. The mechanism of SR-BI-mediated selective lipid uptake has yet to be defined. The clustering of SR-BI in caveolae on the surfaces of cultured cells (35) and in cannaliculi-like structures in the adrenal cortex (ref. 30; perhaps representing microvillar channels; ref. 36) raised the possibility that specialized membrane domains may play a role in the selective uptake process. Because of microscopic reversibility and observations in mice overexpressing SR-BI, the potential of SR-BI for mediating cholesterol efflux from cells was proposed (8) and subsequently demonstrated (37). Its physiological significance has not been established, although it could play a role in reverse cholesterol transport. An alternatively spliced form of SR-BI with an altered C-terminal cytoplasmic domain was discovered by Webb *et al.* (38). This isoform was initially called SR-BI.2 but perhaps should be designated SR-BII to conform to the standard scavenger receptor nomenclature (39). Little is known currently about the physiological consequences of the alternative splicing, but it seems likely that further exploration of this discovery should uncover interesting new insights.

In addition to HDL, SR-BI has been shown to bind to a diverse array of ligands. SR-BI along with another class B scavenger receptor, CD36, were the first anionic phospholipid receptors to be identified (40). This finding suggested that SR-BI might be involved in recognizing senescent or apoptotic cells. Such recognition has been observed *in vitro* (26, 41), but its *in vivo* significance, along with that of SR-BI's binding chemically modified LDL (42), is unclear. Perhaps most relevant to fully describing normal lipoprotein metabolism, SR-BI binds native LDL with high affinity (42, 45), although LDL does not effectively compete with HDL binding (6). Because normal mice have very low steady-state plasma LDL concentrations, it apparently will be necessary to examine the potential physiological relevance of the LDL binding in other species (e.g., rabbit and primates) or in genetically altered murine models (43). It is possible that SR-BI may serve as a backup or alternative receptor to the critically important, classic LDL receptor. Finally, we do not know how HDL exerts its anti-atherosclerotic effects, and thus, it is uncertain whether increasing or decreasing hepatic SR-BI activity might help prevent atherosclerosis. Given the rapid pace of progress in this area in the past two years, it is reasonable to expect resolution of many of these issues in the near future.

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